TLC Determination of Sulfanilamide as a Degradation Product in Pharmaceutical Preparations Containing Sodium Sulfacetamide

By MURRAY P. GRUBER and ROBERT W. KLEIN

Thin-layer chromatography followed by spectrophotometric analysis of eluted components was successfully employed for the separation and determination of sulfanilamide as a degradation product in sulfacetamide preparations. Significant amounts of sulfanilamide were found to be present as a contaminant in a variety of commercially available ophthalmological and dermatological pharmaceuticals containing sulfacetamide, when subjected to accelerated testing conditions and/or prolonged storage. The procedures used may be applicable to stability testing of other sulfonamides.

FOR SEVERAL DECADES, sulfonamides, especially sulfacetamide and its sodium salt, have been widely and successfully employed in the treatment of ocular and dermatological diseases (1–4). Investigators (5, 6) have observed that pharmaceutical preparations containing sulfonamides or their sodium salts undergo a color change on aging, becoming yellow to red-brown, depending on the sulfonamide(s) present.

Various recommendations (5, 6) were made to eliminate the problem of darkening, but they have not proved completely successful. The replacement of air by nitrogen in filled containers and the introduction of small percentages of antioxidants in the finished sulfacetamide products retard but do not prevent discoloration from occurring, particularly on exposure to elevated temperatures, direct sunlight, or prolonged shelf storage.

Since color changes have historically signified some form of chemical reaction ensuing in a substance, this laboratory undertook an exploratory investigation to determine if this phenomenon was indicative of decomposition.

Aqueous solutions ranging from 5 to 30% of sodium sulfacetamide were prepared. The preservative measures previously suggested were employed in an effort to prevent development of the discoloration. All precautionary measures either alone or in combination failed.

The conventional titrimetric methods of analysis as outlined by USP XVII (7) and NF XII (8) were utilized for the quantitative determination of sulfacetamide in these preparations and in each instance, employing these procedures, no decomposition was observed.

Received February 19, 1968, from the Analytical Research Department, Dermik Laboratories, Inc., Syosset, NY 11791
Accepted for publication April 2, 1968.

Although no subpotency was revealed, since the procedure used is nonspecific for sulfonamides, there appeared to be a possibility that the sulfacetamide was undergoing hydrolysis to yield sulfanilamide. In order to pursue this hypothesis, other techniques which would permit the separation of the sulfonamides prior to analysis were essential.

Chromatographic methods have met with some success in the separation of mixed sulfonamides and related materials. Shepherd, Taft, and Krazinski (9) used descending paper partition chromatography in their work on 4-sulfanilamidopyrimidines. The NF XII (8) describes a similar paper chromatrographic procedure for the separation and identification of sulfamerazine and sulfadiazine. Klein and Kho (10), using a modification of the solvent system described by Wollish, Schmall, and Hawrylyshyn (11) obtained satisfactory separations for various sulfonamides including sulfanilamide from sulfacetamide.

Accordingly, a stability evaluation of commercially available preparations was instituted using a TLC adaptation of the developing system described by Shepherd *et al.* (9). Visualization and quantitation of the developed systems was accomplished using Bratton-Marshall reagent or ultraviolet light followed by spectrophotometric determination of the eluted components.

EXPERIMENTAL

Apparatus and Reagents—Precoated 10 × 20-cm. Silica Gel GF plates, 250 and 500 μ thickness (Analtek, Inc., Wilmington, Del.) and a spectrophotometer, Beckman DU or equivalent, equipped with 1-cm. cells were used. Spray reagents, 1 *N* aqueous hydrochloric acid, 5% aqueous sodium nitrite, and *N*-(1-naphthyl)ethylenediamine dihydrochloride

0.1% in 95% ethanol (Bratton-Marshall reagent) were used. Reagents for colorimetric assay were 0.1 *N* aqueous hydrochloric acid, 2.0% aqueous ammonium sulfamate, and 0.4% aqueous sodium nitrite (freshly prepared). Chromatographic jars were equipped for ascending chromatography of sufficient internal volume for 10 × 20-cm. plates. Suitable delivery pipets for spotting purposes were used. Developing solvents system were (*a*) *n*-butanol, A.R. (*b*) ammonia 28%, A.R.

Preparation of Chromatographic Plates—All plates were activated at 40° for 30 min. and stored in a desiccator over silica gel for 1 hr. prior to use.

Standard Preparation—A 50-mg. quantity, accurately weighed, of each sulfonamide was dissolved in 30 ml. of acetone and diluted to 50 ml. with acetone. Sodium sulfacetamide was first dissolved in 1 ml. of distilled water prior to dilution with acetone.

Sample Preparation—A sample equivalent to 50 mg. of sulfacetamide or sodium sulfacetamide was dispersed in approximately 30 ml. of acetone and shaken for 15 min. The mixture was filtered (if not clear) over Whatman No. 1 paper into a 50-ml. volumetric flask and washed with acetone to volume.

Tablets were first crushed to a fine powder using a mortar and pestle and the procedure continued as above.

Sample Size—(*a*) For screening and quantitative determination of major sulfonamide; 10 μl. of sample (equivalent to 10 mcg. of labeled sulfonamide). (*b*) For quantitation of sulfonamide impurities; 50 μl. of sample applied in a band 1 cm. long for glass plates. Larger samples may be spotted if concentrations lower than 1% of sulfonamide impurities are present. Care must be taken that the applied band be as tight as possible.

Developing System—Add 90 ml. of butanol, 10 ml. of concentrated ammonium hydroxide, and 80 ml. of water to a 250-ml. separator. Shake well and allow the phases to separate. The butanol fraction is used as a developing system. Butanol–ammonium hydroxide–water (9:1:8) by volume.

Tank Preparation—All tanks were lined with Whatman No. 1 paper, filled to a depth of 1.2 cm. with developing solvent, and allowed to equilibrate overnight prior to use. The distance developed was 150 mm.

Procedures and Analysis—Samples and standards (sulfacetamide and sulfanilamide) were applied according to the appropriate procedure. After a solvent travel of 150 mm. the plates were removed and air dried. The component spots were observed under a short wavelength ultraviolet light (257 mμ) and marked.

If the screening procedure was being used, the plates were sprayed with 1 *N* hydrochloric acid followed by 5% sodium nitrite. They were then dried for 10 min. at 100°, cooled, and sprayed with 0.1% *N*-(naphthyl)ethylenediamine forming reddish-purple spots for the sulfonamides. The plates were reexamined to determine the presence of a spot produced by sulfanilamide contamination not detected under ultraviolet light.

If quantitation of the sulfacetamide present was desired, the area marked was completely scraped from the plate into a 25-ml. volumetric flask through a small funnel and the area from which the silica gel was removed carefully washed with approximately 20 ml. of 0.1 *N* hydrochloric acid into the flask. This procedure was repeated for subsequent samples, beginning with samples on the outer portion of the plate and working in. The flask and its contents were shaken for 30 min. and diluted to volume with 0.1 *N* hydrochloric acid. A 12.5-ml. aliquot of the clear supernatant was transferred to a 25-ml. flask, 0.25 ml. of freshly prepared 0.4% aqueous sodium nitrite added, and swirled intermittently for 3 min. A 0.25-ml. volume of a 2% aqueous ammonium sulfamate solution was added, the contents gently shaken for 2 min., and followed by the addition of 2.0 ml. of aqueous 0.1% *N*-(naphthyl)ethylenediamine dihydrochloride. The solution was allowed to stand with occasional shaking for 15 min. The absorbance of the solution was determined in a 1-cm. cell at 545 mμ *versus* a reagent blank prepared from a portion of the plate having the same dimensions and no sulfonamide present. The concentration was determined from an absorbance-concentration calibration curve of sodium sulfacetamide obtained using the same procedure. Results obtained from three replicates of the same sample were averaged and calculated as "% of labeled amount" of sodium sulfacetamide.

An alternate procedure involved elution from the silica gel with 4.0 ml. of 95% ethanol, centrifuging the resulting suspension, and determination of the absorbance of the clear supernatant in a 1-cm. cell at 259 mμ *versus* 95% ethanol eluate obtained from a portion of the plate having the same dimensions and no sulfonamide present. Concentration in the eluate was determined using the absorptivity of sodium sulfacetamide in 95% ethanol at 259 mμ. Calculations were carried out and reported as "% of labeled amount."

Determination of sulfanilamide as a decomposition product was carried out in a similar fashion to the determination of sodium sulfacetamide. Due to the smaller quantity of solvent involved in the direct spectrophotometric technique, this procedure in combination with 500-μ plates was preferred. Where comparatively large amounts of sulfanilamide are noted, the procedures are interchangeable. Results were reported as "% of total sulfonamide found."

RESULTS AND DISCUSSION

Analyses were performed on preparations prior to and following chromatographic separation. It was found that the use of precoated plates significantly reduced variation of the R_f values although standards were run concurrently with samples on each plate to determine the experimental range of R_f values and elution recoveries of the sulfonamides present. These figures are noted in Table I. Some varia-

TABLE I—R_f VALUES AND ULTRAVIOLET RECOVERY ANALYSIS

	R_f	Elution Recovery % UV Analysis
Sodium sulfacetamide	0.14	98.4
	0.14	98.8
	0.16	101.4
	$\bar{x}$ = 0.15	$\bar{x}$ = 99.5
Sulfanilamide	0.50	100.2
	0.50	97.8
	0.49	99.6
	$\bar{x}$ = 0.50	$\bar{x}$ = 99.2

TABLE II—STABILITY ANALYSIS OF SULFACETAMIDE SUSPENSIONS AND SOLUTIONS

Sample	Sulfacetamide (% of Labeled Amount) Initial	6 mos. at RT	4 mos. at 40°	Sulfanilamide (% of Total Sulfonamide) Initial	6 mos. at RT	4 mos. at 40°
A	104.6	98.7	93.6	0.4	4.1	7.8
B	98.4	89.5	82.6	1.2	7.3	12.1
C	102.1	96.1	91.2	0.2	4.6	8.0
D	91.0	87.8	80.2	4.6	7.0	13.0
E	97.4	91.0	83.8	2.2	6.2	11.8
F	105.6	103.0	101.7	0	1.5	2.4

tion in sensitivity and lower detection limit was observed between detection techniques. This, however, posed no particular problem in the overall study.

All of the ophthalmic and dermatologic pharmaceutical samples used in the investigation are commercially available. Stability testing was carried out at room temperature for 6 months and at 40° C. (105° F.) for 4 months. The authors felt that this elevated temperature was not unreasonable since this condition is commonly encountered during storage, particularly during the summer months and for longer duration in the southern areas of the country. With the exception of one preparation, wherein the sodium sulfacetamide was packed separately as a dry powder with instructions to the pharmacist for incorporation at the time of dispensing, none bore an expiration date (Table II).

The separately packed sodium sulfacetamide was also analyzed along with tablets containing sulfacetamide at the same temperatures and for the same durations. No deterioration was detected in any of these materials by any method used (Table III).

TABLE III—STABILITY ANALYSIS OF SULFACETAMIDE TABLETS AND POWDER[a]

Sample	Sulfacetamide (% of Labeled Amount) Initial	6 mos. at RT	4 mos. at 40°
Powder A	100.2	100.8	100.7
Powder B	99.6	100.2	99.8
Tablets A	106.2	104.6	106.1
Tablets B	101.2	102.0	101.1

[a] No sulfanilamide was found in the above preparations.

These results confirm the qualitative findings of Klein and Kho (10) who detected unlabeled sulfanilamide in suspension preparations of mixed sulfonamides containing sulfacetamide but not in tablet formulations of sulfacetamide.

Sulfanilamide was the only significant decomposition product found in sulfacetamide preparations. This was only evident after chromatographic separation. In addition, the amount of sulfanilamide present increased with length of storage. The preparation containing separately packaged dry sulfacetamide powder exhibited degradation characteristics similar to those in the same classification on prolonged storage. However, this pattern emerged only after incorporation of the sodium sulfacetamide into the vehicle and the completed preparation remained stable for the period indicated on the package insert.

SUMMARY

The customary analytical techniques as outlined in USP XVII and NF XII were demonstrated to be inadequate for purity and stability testing of pharmaceutical formulations containing sulfacetamide or its sodium salt.

Pharmaceutical suspensions and solutions containing sodium sulfacetamide were tested *via* TLC for stability under conditions of elevated temperature and prolonged storage at room temperature. All showed significant degradation to sulfanilamide. However, tablets and dry sodium sulfacetamide powder appear to be stable. Since sulfanilamide is a primary sensitizer, occurrence of sensitivity arising from prolonged use of sulfacetamide may be due to the concomitant presence of sulfanilamide as a contaminant.

This study demonstrates that ophthalmological and dermatological preparations of sulfacetamide and its sodium salt have a limited shelf life and should bear an expiration date. However, the possibility that other sulfonamides undergo similar degradation is a consideration which bears further investigation.

Methods for the quantitation of stability data for sulfacetamide are proposed which appear applicable to varied preparations of mixed sulfonamides.

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Keyphrases

Sulfacetamide solutions—stability
Sulfanilamide formation—sulfacetamide solutions
TLC—separation, identity
UV spectrophotometry—analysis
Colorimetric analysis—spectrophotometry