which agrees satisfactorily with the sum of 107.0% found separately. The presence of a metal–I complex of this type is indicated whenever the residue resulting from the evaporation of the chloroform fraction does not completely dissolve in the alcohol solvent used for the determination of the corticosteroid.

The presence of a cyclohexane-soluble metal complex of I is indicated when the amount of I cannot be determined by the UV procedure due to interference in the absorbance at 326 nm and when there is an absorbance peak close to 406 nm in the UV spectra. Sample 19 is an example of a formulation that contains both types of metal–I complexes. Analysis of a single tube composite by the proposed procedure indicated the presence of 88.1% by the UV method and 98.1% by the complexiметric method. The chloroform fraction was treated in the same manner as described for Sample 8 and was found to contain 46.1% of the amount of I declared. The total complexed and free I was determined by dissolving a second sample of the composite in chloroform, adding the nickel reagent, and scanning against a blank of the nickel reagent in chloroform. Total I was determined to be 149.8%, which compares satisfactorily with the 144.2% found by summing the values obtained separately.

Chloroform is not the solvent of choice for the determination of I by the complexiметric method since the nickel complex can exist in two different forms in chloroform; one absorbs at 402 nm and one absorbs at 470 nm. The equilibrium between these forms is affected greatly by the water concentration and causes the measurement at 402 nm to be lower than it should be whenever any water is present. The chloroform fraction is used to estimate the amount of the cyclohexane–insoluble metal–I complex since the solid metal–I complex remaining from the evaporation of the chloroform eluate was not sufficiently soluble in the 13 solvents listed in Table II. In the early phases of the investigation, the cyclohexane fraction was evaporated on a steam bath and the residue was dissolved in chloroform for the complexiметric determination. The trouble with the equilibrium between the two complex forms and the volatility of I led to the development of the direct measurement in the cyclohexane eluate.

The results for Samples 4, 7, 8, 19, 22, and 23 that were run on different days and/or from different containers did not agree satisfactorily, even though duplicates run at the same time were in close agreement for both I and the corticosteroid. This finding indicates nonuniformity in mixing during preparation and/or packaging.

Several products tested contained other pharmaceutically active ingredients such as lidocaine, neomycin sulfate, and pramoxine hydrochloride. No attempt was made to determine these components quantitatively.

Sample preparations and column elution for two samples require approximately 1.5 hr, and complete determination by all four determinative procedures can be completed in approximately 6 hr.

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# Assay of Sulfacetamide Sodium Ophthalmic Solutions by High-Pressure Liquid Chromatography


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**Abstract** □ A high-pressure liquid chromatographic method, using an adsorption column and sulfabenzamide as the internal standard, is proposed for the determination of sulfacetamide sodium and its principal hydrolysis product, sulfanilamide, in eye drops. It affords an average recovery of 100.9% of added sodium sulfacetamide with a relative standard deviation of 1.9%.



**Keyphrases** □ Sulfacetamide sodium—ophthalmic solutions, assay, high-pressure liquid chromatography □ Sulfanilamide—assay as hydrolysis product of sulfacetamide sodium in ophthalmic solutions, high-pressure liquid chromatography □ High-pressure liquid chromatography—assay of sulfacetamide ophthalmic solutions


Sulfacetamide sodium solutions have been shown to undergo hydrolysis to sulfanilamide and sodium acetate and oxidative discoloration (1–5). The nitrite titration method used for sulfacetamide sodium ophthalmic solution in USP XVIII (6) does not distinguish between sulfacetamide and sulfanilamide

**Table I**—Response Factors for Standard Solutions[a]

| Sulfonamide | Average Response Factor | *SD* | Variance | Coefficient of Variation, % |
|---|---|---|---|---|
| Sulfanilamide | 6.4939 | ±0.2575 | 0.0663 | ±4.0 |
| Sulfacetamide | 0.8474 | ±0.0123 | 0.0001 | ±1.5 |

[a] Data calculated from three to five injections of each of four standard solutions.

**Table II**—Recovery of Sulfacetamide Sodium Added in Ophthalmic Preparations

| Formulation | Recovery, % | Average Recovery, % | *RSD*, % |
|---|---|---|---|
| A | 102.0, 100.3, 102.7, 102.3, 101.7, 100.0, 102.3, 102.0, 102.0, 102.0 | 101.7 | ±0.9 |
| B | 101.0, 101.3, 102.3, 104.3, 102.0, 102.7, 104.3, 100.0 | 102.2 | ±1.4 |
| C | 98.3, 96.7, 97.3, 99.3, 99.3, 99.0, 99.3, 100.3 | 98.7 | ±1.1 |

and is thus not stability indicating with respect to hydrolytic decomposition. A colorimetric assay was suggested based on the reaction of sulfacetamide sodium, an imide, with hydroxylamine and ferric chloride to form the ferric acethydroxamate complex (7). A quantitative TLC method was proposed in which sulfacetamide was separated from its degradation products with butanol–ammonia–water (9:1:8) followed by quantitation of the eluted zones *via* the Bratton–Marshall reaction (8). An alumina adsorption column and UV spectrophotometry were utilized for the separation and determination of sulfanilamide in ophthalmic solutions and tablets containing sulfacetamide (9); sulfacetamide is retained on the column.

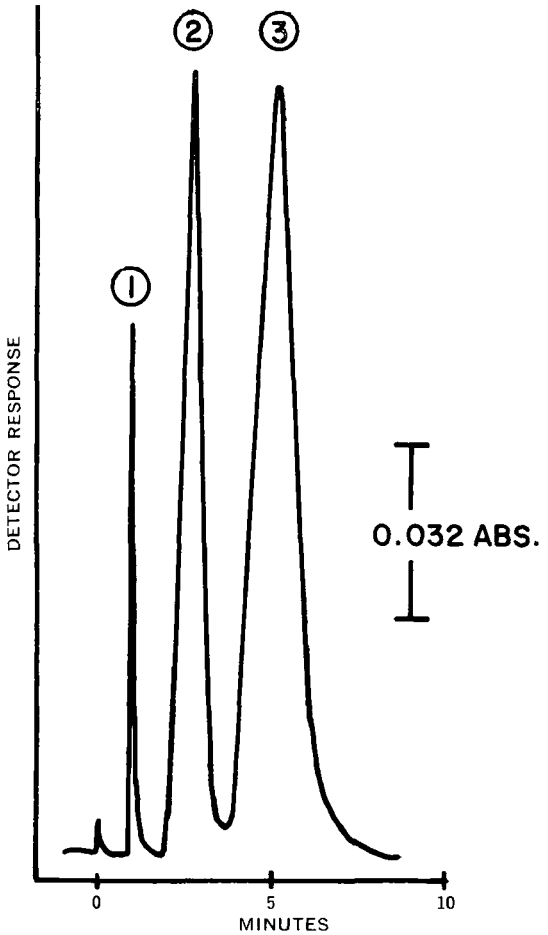


**Figure 1**—*Liquid chromatographic separation of the three sulfonamides. Key: 1, sulfanilamide; 2, sulfabenzamide (internal standard); and 3, sulfacetamide sodium.*

The recent availability of instrumentation for high-pressure liquid chromatography and reports (10, 11) on separations of sulfa drug mixtures using this technique with ion-exchange resins stimulated the work described here. A simple method was developed which requires only dilution of the sample to incorporate the internal standard and provides complete separation and quantitation of sulfacetamide and sulfanilamide within 10 min on an adsorption column. The method was used in a survey of the stability of several commercial sulfacetamide sodium formulations.

## EXPERIMENTAL

**Apparatus**—A liquid chromatograph composed of a pump[1] (3000 psi maximum) with a pulse dampener and a UV monitor[2] (254 nm), a septumless injector[3], and a strip-chart recorder[4] outfitted with a potentiometric amplifier was used. A 70-cm × 2-mm i.d. stainless steel column packed with Corasil II, 37–50-μm particle size, was operated at 400 psi, with a 65% pump stroke resulting in a flow rate of about 2 ml/min. The mobile phase employed consisted of methylene chloride–isopropanol–concentrated ammonia (130:65:2.5), which was degassed by gentle heating prior to use. All injections were made with a 25-μl syringe[5].

**Standard Preparation**—Prepare individual stock solutions of sulfanilamide and sulfabenzamide internal standard at concentrations of 0.3 and 1.5 mg/ml of isopropanol, respectively. Accurately prepare a series of standard curve solutions to contain 0.9–1.65 mg of sulfacetamide sodium plus 0.15–1.5 mg of sulfanilamide/ml in 90% isopropanol. Each standard solution should contain 6.0 mg of internal standard/ml. The concentrations of sulfonamides represent a range of 60–110% sulfacetamide sodium and 1–10% sulfanilamide.

**Sample Preparation**—Dilute the sample solutions with isopropanol to obtain a concentration of 15 mg/ml. Pipet 1 ml of each sample into a 10-ml volumetric flask, add 4.0 ml of internal standard solution, and dilute to volume with isopropanol.

[1] Milton Roy.
[2] LDC.
[3] Varian.
[4] Heath.
[5] Precision Sampling Pressure-Lok liquid, Pierce Chemical Co.

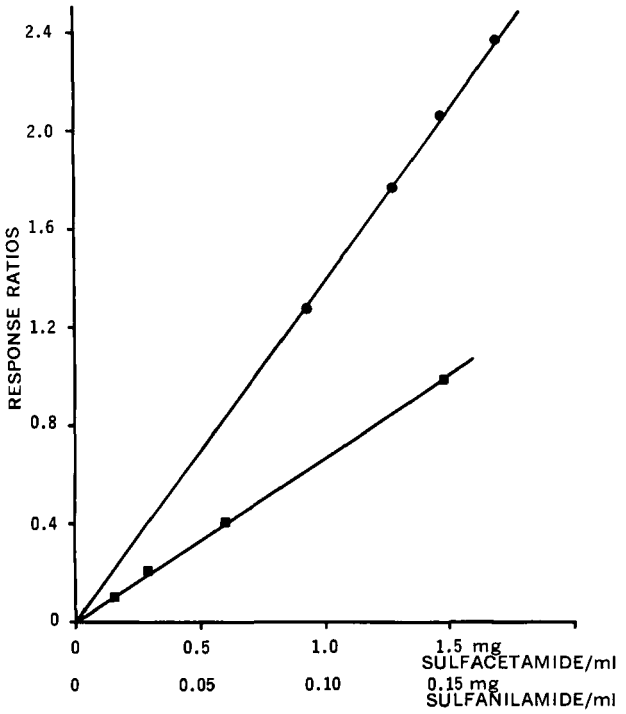


**Figure 2**—*Calibration curves indicating linearity of response of UV detector to sulfanilamide* (■) *and sulfacetamide sodium* (●).

**Chromatographic Separation**—With the UV monitor set at a suitable sensitivity range, inject 10 μl of each standard solution in duplicate. Sulfanilamide, sulfabenzamide, and sulfacetamide sodium appear as peaks after 1.0, 2.5, and 5.0 min, respectively. Compute the area of the sulfabenzamide and sulfacetamide sodium peaks arising from each standard injection, and determine the peak height of the sulfabenzamide and sulfanilamide peaks in each standard injection. Calculate the response factor, $R$, for sulfanilamide and sulfacetamide.

Inject each sample solution in duplicate, and calculate the concentrations of sulfacetamide sodium and sulfanilamide, if present, using the average response factors determined from the standards.

## RESULTS AND DISCUSSION

All chromatographic runs were made employing a UV detector sensitivity of 0.32 absorbance unit full scale. A typical chromatogram is presented in Fig. 1. An internal standard was used to minimize the effects of small procedural variations. Sulfabenzamide was chosen as the internal standard since it eluted between the two sulfonamides of interest and, therefore, would not increase the analysis time.

The output of the UV detector was fed *via* an interface to a computer[6] programmed to determine peak heights and areas. Since sulfanilamide eluted as a rather narrow sharp peak, peak height rather than area was chosen as a more accurate measure of the concentration of this component. For the quantitation of intact sulfacetamide sodium, peak areas were employed. A linear response was obtained for the undegraded drug and its decomposition product over concentration ranges of 0–1.7 and 0–0.15 mg/ml, respectively (Fig. 2).

[6] IBM System 7.

**Table III**—Results of Replicate Assays of Sulfacetamide Sodium Ophthalmic Solutions

| Solution | Sulfacetamide Sodium Found, % | Theory, % | Percent of Label Claim | Sulfanilamide, % |
|---|---|---|---|---|
| A | 29.9 | 30.0 | 99.7 | 0.7 |
| B | 29.6 | 30.0 | 98.7 | 1.8 |
| C | 30.0 | 30.0 | 100.0 | 2.9 |
| D | 15.3 | 15.0 | 102.0 | 1.0 |
| E | 14.4 | 15.0 | 96.0 | 3.9 |
| F | 13.7 | 15.0 | 91.3 | 6.5 |
| G | 13.0 | 15.0 | 86.7 | 8.0 |
| H | 9.3 | 10.0 | 93.0 | 1.9 |
| I | 9.4 | 10.0 | 94.0 | 4.1 |
| J | 10.5 | 10.0 | 105.0 | 0.9 |

To compensate for day-to-day variations in any operating parameter, response factors for each sulfonamide were determined from a limited number of standard solutions each day the samples were analyzed. Since the response factors were linearly related to concentration, average response factors for sulfanilamide and sulfacetamide were calculated (Table I).

The results of adding known amounts of sulfacetamide sodium to each of three analytically prepared test formulations are shown in Table II. The data were obtained with replicate aliquots of each formulation injected in duplicate. Pooling the results of all 26 assays resulted in an average recovery of 100.9% with a relative standard deviation of 1.9%.

Sulfacetamide sodium ophthalmic preparations are usually available at solution concentrations of 30, 15, and 10%. Three different formulations[7] were assayed for each concentration, and two different lots of the same preparation were tested in one instance. The average results obtained for replicate aliquots of each formulation are presented in Table III. The only degradation product detected was sulfanilamide.

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[7] The formulations used were marketed preparations. None of the preparations with expiration dates was outdated.