Colorimetric Determination of Acetyl Sulfisoxazole in the Presence of Its Hydrolysis Products, Sulfisoxazole and Acetic Acid

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Abstract □ The official assay for acetyl sulfisoxazole, the diazotization method, does not differentiate between acetyl sulfisoxazole and its main hydrolysis product, sulfisoxazole. A spectrophotometric method, involving the formation of ferric acetohydroxamate, was developed, and conditions necessary for reproducible reaction were developed. The method provides a relatively simple and rapid means by which acetyl sulfisoxazole is quantitatively determined in the presence of its main hydrolysis products, sulfisoxazole and acetic acid.

Keyphrases □ Acetyl sulfisoxazole—colorimetric determination in the presence of sulfisoxazole □ Ferric acetohydroxamate—formation in the colorimetric determination of acetyl sulfisoxazole □ Colorimetry—determination, acetyl sulfisoxazole

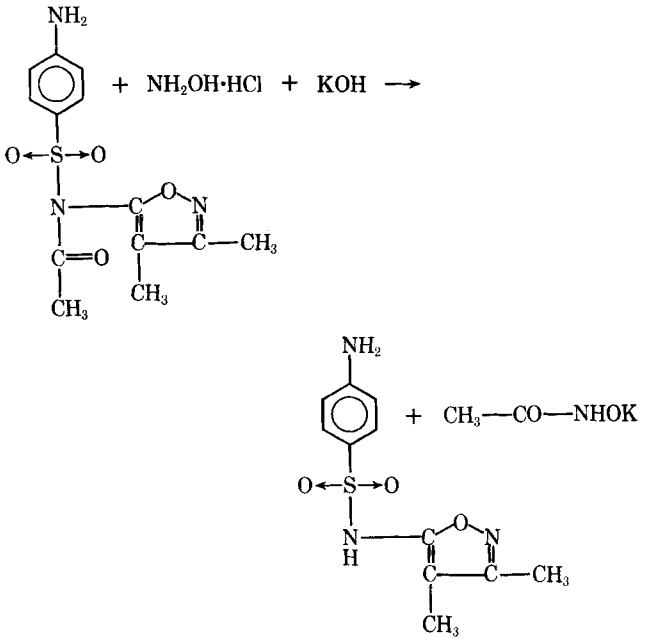
Acetyl sulfisoxazole NF is a white crystalline powder, m.p. 192–195°. Acetyl sulfisoxazole is practically insoluble in water, slightly soluble in alcohol, and sparingly soluble in chloroform. Chemically, it is N^1-acetyl-N^1-(3,4-dimethyl-5-isoxazolyl)sulfanilamide, and it is prepared by the acetylation of a solution or suspension of the alkali metal salt of N^1-(3,4-dimethyl-5-isoxazolyl)sulfanilamide (sulfisoxazole) (1, 2).

The analytical procedure presently official in NF XIII (3) is based on the diazotization of the primary amino group attached to the aromatic ring and the determination of the end-point electrometrically. Since this functional group is present in both sulfisoxazole and acetyl sulfisoxazole, this method of analysis cannot be used to differentiate between the two sulfonamides.

Acetyl sulfisoxazole has been analyzed by diazotization, X-ray diffractometry of the oral suspension (4), and the Bratton and Marshall method as adapted by Flake *et al.* (5) and Hagler *et al.* (6). Neither the cited procedures nor the official method allow for the direct quantitative analysis of acetyl sulfisoxazole in the presence of sulfisoxazole. Bican-Fister and Kajganovic (7) reported the application of TLC to the separation of sulfonamide mixtures, but this method is useful qualitatively rather than quantitatively.

The most common colorimetric method for the analysis of sulfonamides is the method of Bratton and Marshall. This method involves the diazotization of sulfonamide with sodium nitrite in dilute acid, followed by decomposition of the excess nitrite with sulfamic acid and coupling of the diazo compound with *N*-(1-naphthyl)ethylenediamine. The important disadvantage of this method and other colorimetric methods reported in the literature (8–12) is that the absorption frequencies and molar absorbance coefficients produced with acetyl sulfisoxazole and sulfisoxazole are identical. Therefore, these methods are not differential.

In an attempt to find a specific analysis that would be both quantitative and differential between acetyl sulfisoxazole and sulfisoxazole, the method of Feigl *et al.* (13) was selected. By this method, carboxylic acid derivatives (acid anhydrides, acid halides, and esters) are converted to corresponding hydroxamic acid salts by allowing them to react with hydroxylamine hydrochloride in an alkaline medium. The hydroxamic acid salt is then allowed to react with ferric chloride in the presence of dilute acid to produce a red-violet ferric hydroxamate. Soloway and Lipschitz (14) reported that amides can be converted into hydroxamic acid salts with hydroxylamine. The hydroxaminolysis of amides and the formation of colored complexes of the hydroxamic acids so derived with ferric ion afford convenient means of determining amides in the presence of their amino compounds and acid constituents. A procedure for the quantitative analysis of sodium sulfacetamide, which is the sodium salt of N^1-acetylsulfanilamide, involves the formation of ferric acetohydroxamate and measurement of the absorbance at the 540-nm. wavelength, as reported by Schleider *et al.* (15). This method permits the determination of intact sulfacetamide in the presence of its hydrolysis products, sulfanilamide and sodium acetate.



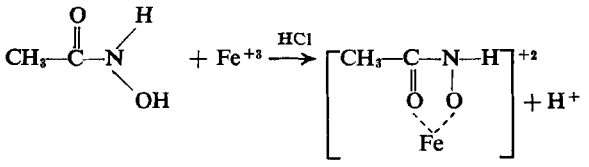
Scheme I

Acetyl sulfisoxazole, a sulfonamide acetylated at the N^1-position, could conceivably react with hydroxylamine hydrochloride in alkaline medium to form the acetohydroxamic acid salt, as shown by Scheme I. The acetohydroxamic acid salt produced could then react with ferric ion in acidic medium (pH 0.5–1.5)

Table I—Effect of Temperature on Color Produced (Absorbance)

Acetyl Sulfisoxazole, mg.	At Ice Bath Temperature	At Room Temperature	At Boiling Water Bath Temperature
0.0	0.030	0.030	0.030
1.0	0.375	0.370	0.370
2.0	0.690	0.685	0.700

to produce the colored ferric hydroxamate according to Scheme II (16).



Scheme II

EXPERIMENTAL

Apparatus—The following were used: a colorimetric spectrophotometer[1], and one set (12) of standard matched test tube cells, i.d. 11.67 mm[2].

Reagents—The following were used: acetyl sulfisoxazole[3], sulfisoxazole[3], acetic acid A.R., sodium acetate A.R., potassium hydroxide A.R., hydroxylamine hydrochloride[2], isopropanol A.R., methanol A.R., ferric chloride A.R., hydrochloric acid A.R., and distilled water.

Solutions—The following were used: saturated hydroxylamine hydrochloride solution (prepared by dissolving 83 g. in 100 ml. of distilled water), 10.5 *M* potassium hydroxide, 4.1 *M* hydrochloric acid solution (50% v/v), isopropanol (33.3% v/v), 0.37 *M* ferric chloride solution in 0.1 *M* hydrochloric acid, and 0.2% w/v methanolic solution of acetyl sulfisoxazole. The solutions were stored under refrigeration and allowed to come to room temperature prior to use.

The proposed method was applied to solutions containing acetyl sulfisoxazole, sulfisoxazole, acetic acid, and sodium acetate; it was found that the color was produced exclusively by acetyl sulfisoxazole. The effects of temperature, quantity of reagents, and reaction time for optimum color formation and rate of color fading were determined.

Since acetyl sulfisoxazole is practically insoluble in water, a methanolic solution of acetyl sulfisoxazole was used. Transfer 0–1-ml. portions of the methanolic solution of acetyl sulfisoxazole into test tubes, and add sufficient quantity of methanol to adjust the volume to 1 ml. To each tube, add 3 ml. of the isopropanol solution, followed by the addition of 1 ml. of the hydroxylamine hydrochloride solution and 1 ml. of the potassium hydroxide solution. Mix the solutions, and allow the reaction to proceed for the specified time interval. Then add 2 ml. of the hydrochloric acid solution. Check the pH of the resulting solution to ascertain whether the pH is between 0.5 and 1.5. If the pH is higher than 1.5, adjust by addition of one or two drops of concentrated hydrochloric acid. Add 1 ml. of the ferric chloride solution and shake. Measure the absorbance of the solution immediately at a wavelength of 540 nm.

The absorbance was plotted as a function of concentration of acetyl sulfisoxazole. A straight-line relationship was observed for a 0–2-mg. concentration of acetyl sulfisoxazole, indicating adherence to Beer's law over this range.

DISCUSSION

In the studies of the rate of reaction of acetyl sulfisoxazole with hydroxylamine hydrochloride, the instantaneous reaction was observed at room temperature. The minimum amounts of reagents necessary for the maximum color production in the systems containing 2 mg. or less of acetyl sulfisoxazole were determined. The Lambert–Beer curve was plotted, and the results were reproducible.

[1] Spectronic-20, Bausch & Lomb, Inc.
[2] Fisher Scientific Co.
[3] Hoffmann–La Roche, Inc.

Table II—Effect of Reaction Time on Color Produced[a]

Sample	Minutes	Absorbance
1	0.0	0.520
2	15	0.515
3	30	0.520
4	45	0.530
5	60	0.525
6	75	0.515

[a] A blank for each sample gave 0.000 absorbance.

Effect of Temperature Applied after Addition of Hydroxylamine Hydrochloride on Absorbance—A series of triplicate test tubes, containing 0.0, 0.5, and 1.0 ml. of the acetyl sulfisoxazole solution, was prepared. To each tube, isopropanol solution, hydroxylamine hydrochloride solution, and potassium hydroxide solution were added as described previously. One set of tubes was allowed to remain at room temperature for 1 hr.; the other two sets were kept at ice bath and boiling water bath temperatures, respectively, for 1 hr. The contents of each tube were then brought to room temperature and acidified with hydrochloric acid solution; the ferric chloride solution was added as previously described. The absorbance of the solutions was measured at 540 nm. (Table I).

Effect of Varying Length of Time after Addition of Hydroxylamine on Absorbance—A sample of 0.75 ml. of 0.2% acetyl sulfisoxazole solution was placed into each of six test tubes. The isopropanol solution, hydroxylamine hydrochloride solution, and potassium hydroxide solution were added as described previously. The tubes were set aside at room temperature for 15, 30, 45, 60, and 75 min., respectively. As each sample was removed, hydrochloric acid and ferric chloride solutions were added as described. Blanks, corresponding to each time interval, were also prepared. The absorbances were then determined at a wavelength of 540 nm. (Table II).

Effect of Various Concentrations of Acetyl Sulfisoxazole on Absorbance—Samples of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 ml. of the 0.2% methanolic solution of acetyl sulfisoxazole, containing the equivalent of 0–2 mg. of acetyl sulfisoxazole, respectively, were placed in test tubes, and sufficient methanol was then added to each tube to adjust the volume to a total of 1 ml. Three milliliters of the isopropanol solution, 1 ml. of the hydroxylamine hydrochloride solution, and 1 ml. of potassium hydroxide solution were then added in the stated order to the sample. To each tube, 2 ml. of 4 *M* hydrochloric acid solution was added, and the pH was checked. Then 1 ml. of ferric chloride solution (0.3 *M* in 0.1 *N* HCl) was added, and the absorbance was determined at 540 nm. Three analyses were performed upon each of the concentrations under identical conditions of time and temperature (Table III).

Rate of Color Fading of Ferric Complex—One-milliliter samples of a methanolic solution of acetyl sulfisoxazole, containing 1.50 mg. of acetyl sulfisoxazole/ml., were used to produce the reference color in the manner described previously. The colored solutions produced were allowed to stand at room temperature, and the absorbance of samples of this solution was measured at intervals for a total of 90 min. elapsed time (Table IV).

Effects of Altering Reaction Conditions on Absorbance—The following results were observed when isolated changes in the previously stated procedure were investigated.

Table III—Effect of Concentration of Acetyl Sulfisoxazole on Absorbance[a]

Acetyl Sulfisoxazole, mg.	Absorbance (Average of Three Runs)
0.0	0.000
0.2	0.080
0.4	0.105
0.6	0.235
0.8	0.315
1.0	0.405
1.2	0.470
1.4	0.560
1.6	0.645
1.8	0.710
2.0	0.790

[a] Reproducibility of individual values was within the limitation of the spectrophotometer employed.

Table IV—Rate of Color Fading of Ferric Complex[a]

Minutes	Absorbance
0	0.560
1	0.560
2	0.550
3	0.550
4	0.545
5	0.545
6	0.545
7	0.540
8	0.540
9	0.540
10	0.540
11	0.535
12	0.530
13	0.530
14	0.520
15	0.520
30	0.500
45	0.480
60	0.465
75	0.450
90	0.440

[a] A blank for each sample was adjusted for 0.000 absorbance.

When the isopropanol solution was replaced with distilled water, the absorbance value for a 1-mg. sample of acetyl sulfisoxazole decreased from 0.405 to 0.28.

When the potassium hydroxide solution was replaced with distilled water, the absorbance value for a 1-mg. sample of acetyl sulfisoxazole decreased from 0.400 to 0.010, thereby showing essentially no reaction.

When the order of addition of the hydroxylamine hydrochloride solution and the potassium hydroxide solution was reversed, the absorbance value for a 1-mg. sample of acetyl sulfisoxazole decreased from 0.405 to 0.015.

When the amounts of the hydroxylamine hydrochloride solution, potassium hydroxide solution, and ferric chloride solution were increased, the absorbance values for a 1-mg. sample of acetyl sulfisoxazole were identical within experimental variation.

Quantitative Determination of Acetyl Sulfisoxazole in the Presence of Its Hydrolysis Products—The results shown in Table V were observed when the stated procedure was applied to acetyl sulfisoxazole alone and in an admixture containing its hydrolysis products. The values reported represent the average value of four determinations. The absorbance value of 0.470 for 1.5 mg. acetyl sulfisoxazole/10 ml. compares favorably to the absorbance value of 0.520 for 1.5 mg. acetyl sulfisoxazole/9 ml. volume.

CONCLUSIONS

A procedure allowing the quantitative analysis of acetyl sulfisoxazole was developed. The conditions necessary for optimum and reproducible results were established.

The procedure consists of diluting a 1-ml. sample of a methanolic solution of acetyl sulfisoxazole with 3 ml. of aqueous isopropanol solution (33.3% v/v). To this assay preparation, 1 ml. of saturated hydroxylamine hydrochloride solution (83 g. in 100 ml.) and 1 ml. of potassium hydroxide solution (10.5 *M*) are added and mixed. The reaction is instantaneous at room temperature. Subsequently, 2 ml. of hydrochloric acid solution (4.1 *M*) is added to acidify the solution to a pH between 0.5 and 1.5; then 1 ml. of ferric chloride solution (0.37 *M* in 0.1 *N* HCl) is added, and the solution is shaken. The absorbance of each sample is then determined at a wavelength of 540 nm. A straight-line relationship passing through the origin was observed in the plot of absorbance as a function of the initial concentration of acetyl sulfisoxazole over a range from 0 to 2 mg. of acetyl sulfisoxazole/4 ml. of assay preparation.

Table V—Quantitative Determination of Acetyl Sulfisoxazole in the Presence of Its Hydrolysis Products

Concentration/10 ml. Total Volume	Absorbance
Acetyl sulfisoxazole, 1.5 mg.	0.475
Acetyl sulfisoxazole, 1.5 mg., plus sulfisoxazole, 5 mg., and acetic acid, 3–4 mg.	0.470
Acetyl sulfisoxazole, 1.5 mg., plus sulfisoxazole, 5 mg., and sodium acetate, 4.95 mg.	0.470

SUMMARY

Known methods of analysis were examined for application to the quantitative determination of acetyl sulfisoxazole in the presence of sulfisoxazole. The official diazotization method does not differentiate between these two sulfonamides. Therefore, a spectrophotometric (colorimetric) method, involving the formation of ferric acetohydroxamate, was developed and employed. The effects of temperature, reaction time, quantity of reagents, and concentration of acetyl sulfisoxazole on the development of color were examined. The stated method provides for a relatively simple and rapid means by which the concentration of acetyl sulfisoxazole is quantitatively determined in the presence of its main hydrolysis product, sulfisoxazole.

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