Direct Spectrophotometric Determination of Sulfathiazole in Presence of Sulfadiazine and Sulfamerazine

LAWRENCE J. DOMBROWSKI ˣ, ROBERT S. BROWNING, and EDWARD L. PRATT

Abstract □ A direct spectrophotometric method for the quantitative determination of sulfathiazole and the total pyrimidyl sulfonamide content in a mixture containing sulfadiazine and sulfamerazine is reported. This three-component system was readily reduced to the problem of a simple two-component system analysis. Based on dual isoabsorptive wavelength spectroscopy, simultaneous equations were developed that required absorbance measurements at only two selected wavelengths, both isoabsorptive. The location of the isoabsorptive wavelengths was dependent on the pH of the solvent medium, and 0.1 *M* HCl gave the best results. The validity of the derived equations was demonstrated in a recovery study involving synthetic mixtures containing varying quantities of the three sulfonamides. The recovery was linear over a wide concentration range, and the precision of the method was about 1%.

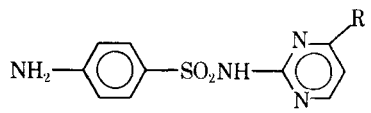
Keyphrases □ Sulfathiazole—spectrophotometric analysis in mixtures with sulfadiazine and sulfamerazine □ Spectrophotometry—analysis, sulfathiazole in mixtures with sulfadiazine and sulfamerazine □ Sulfonamides—sulfathiazole, spectrophotometric analysis in mixtures with sulfadiazine and sulfamerazine □ Antibacterials—sulfathiazole, spectrophotometric analysis in mixtures with sulfadiazine and sulfamerazine

Previous quantitation methods for sulfonamides in mixtures include paper chromatography (1, 2), precipitation followed by UV absorbance measurement (3), complexometric titration (4), partition chromatography, TLC, and GLC (5–10). Most of these procedures require separation of the individual sulfonamides and are time consuming.

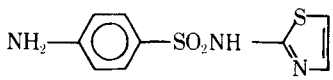
BACKGROUND

A direct spectrophotometric procedure was investigated to determine sulfonamides in combination more rapidly. Quantitative spectrophotometric analysis of two components in one system generally has been simplified by the use of simultaneous equations (11, 12). This process requires that absorbance measurements be made at two wavelengths, λ_1 and λ_2, chosen so that the absorptivity ratio, a_1/a_2, is a maximum at λ_1 and a minimum at λ_2. Knowledge of the four absorptivity values then permits the determination of both components in an unknown. Dual wavelength measurements were used to determine the relative concentration of two components by a graphical absorbance–ratio method (13). This method was greatly simplified when one selected wavelength was isoabsorptive, *i.e.*, a wavelength where numerical values of the individual absorptivities are equal. However, application of direct spectrophotometric analysis to three-component systems generally has not found much use because it requires absorbance measurements at wavelengths where the absorptivity ratios for the various components differ substantially. Therefore, this type of analysis is limited, in part, by the width of the individual absorption band. In addition, there is almost a complete lack of true three-component systems with isoabsorptive points.

Both sulfamerazine (I) and sulfadiazine (II) in 0.1 *M* HCl exhibit two isoabsorptive points at 239 and 279 nm, respectively. By using these analytical wavelengths, it was possible to determine the content of sulfathiazole (III) in a mixture containing all three sulfonamides without interference. Also, the sum total concentration of sulfamerazine and sulfadiazine was simultaneously obtained. Based on dual isoabsorptive wavelength spectroscopy, equations for the three-component system were readily reduced to equations for a two-component system, which greatly simplified the analysis. The method is illustrated by recoveries from a series of solution mixtures containing varying proportions of the three sulfonamides.



I: R = CH_3
II: R = H



III

In certain cases where complete analyses are not required, the direct method based on dual isoabsorptive wavelength spectroscopy may be simple to use.

EXPERIMENTAL

Chemicals and Reagents—All sulfonamides were USP or NF grade. Acetate buffer was prepared by dissolving 27.3 g of sodium acetate trihydrate in 1000 ml of water and adjusting the pH to 6.0 with acetic acid.

Instrumentation—UV spectra of sulfamerazine[1], sulfadiazine[1], and sulfathiazole[2] were obtained using a recording spectrophotometer[3] with matched 1-cm cells.

Preparation of Solutions—Individual stock solutions of I, II, and III were prepared to contain 100.0 μg of drug/ml of 0.1 *M* HCl. Various aliquots of each stock solution ranging from 1.0 to 6.0 ml were transferred to 100-ml volumetric flasks, diluted to volume with 0.1 *M* HCl, and mixed (Table I). The total sulfonamide concentration in the final dilution was between 10.0 and 12.0 μg/ml, and the sulfathiazole concentration was between 1.0 and 5.0 μg/ml.

Analysis of Tablets—A quantity of the finely powdered tablet mixture, equivalent to 100 mg of total sulfonamides, was transferred to a 250-ml erlenmeyer flask. Exactly 100 ml of 0.1 *M* HCl was added, and the flask was shaken mechanically for 15 min to dissolve the sulfonamides. The solution was filtered, and 2.0 ml of the filtrate was transferred to a 100-ml volumetric flask and diluted to volume with 0.1 *M* HCl. Its absorbance was measured at 239 and 279 nm with 0.1 *M* HCl as a reference.

RESULTS AND DISCUSSION

Figure 1 shows the spectra of sulfadiazine (curve D) and sulfamerazine (curve M), which have two isoabsorptive points located at 239 and 279 nm, respectively. The spectrum for sulfathiazole is represented by curve T. At either one of these analytical wavelengths, Beer's law for a mixture containing the three sulfonamides may be written in general as:

$$A_{obs} = b(a_1c_1 + a_2c_2 + a_3c_3) \qquad \text{(Eq. 1)}$$

where c_1, c_2, and c_3 are the sulfamerazine, sulfadiazine, and sulfathiazole concentrations, respectively.

At both isoabsorptive wavelengths, the absorptivities are equal ($a_1 = a_2 = a_{iso}$) and the sum total concentration of the isoabsorptive species is $c_t = c_1 + c_2$. Consequently, the three-component system is reduced to a two-component system with:

$$A' = b(a'_{iso}c_t + a_3'c_3) \text{ at } 239 \text{ nm} \qquad \text{(Eq. 2)}$$

[1] Lederle Laboratories, Pearl River, N.Y.
[2] Eli Lilly, Indianapolis, Ind.
[3] Perkin-Elmer model 323.

Table I—Preparation of Sulfonamide Mixtures

Sample	Aliquots of Stock Solution, ml I	II	III	Total Sulfonamide Concentration in Final Dilution, μg/ml
1	3.0	3.0	4.0	10.0
2	3.0	4.0	3.0	10.0
3	4.0	3.0	3.0	10.0
4	3.0	3.0	4.0	10.0
5	2.0	4.0	4.0	10.0
6	4.0	2.0	4.0	10.0
7	4.0	4.0	2.0	10.0
8	5.0	4.0	1.0	10.0
9	5.0	3.0	3.0	11.0
10	3.0	5.0	3.0	11.0
11	3.0	3.0	5.0	11.0
12	4.0	4.0	3.0	11.0
13	5.0	5.0	1.0	11.0
14	4.0	4.0	4.0	12.0
15	5.0	5.0	2.0	12.0
16	5.0	2.0	5.0	12.0
17	2.0	5.0	5.0	12.0
18	3.0	4.0	5.0	12.0
19	5.0	6.0	1.0	12.0

and:

$$A'' = b(a''_{iso}\, c_t + a_3'' c_3) \text{ at } 279 \text{ nm} \qquad \text{(Eq. 3)}$$

Equations 2 and 3 can be used to determine the actual concentration of sulfathiazole and the total concentration of sulfadiazine and sulfamerazine. These simultaneous equations, when solved, yielded:

$$c_3 = -\frac{(a'_{iso}A'' - a''_{iso}A')}{(a''_{iso}a_3' - a_3''a'_{iso})} \qquad \text{(Eq. 4)}$$

and:

$$c_t = \frac{(a_3'A'' - a_3''A')}{(a''_{iso}a_3' - a_3''a'_{iso})} \qquad \text{(Eq. 5)}$$

To test the validity of Eqs. 4 and 5, the absorbance values at 239 and 279 nm were obtained for the series of mixed solutions listed in Table I. For the calculations, the experimentally derived absorptivity[4] values for each sulfonamide in 0.1 *M* HCl at λ_1 and λ_2 were employed. The λ_1 and λ_2 values were 57.1 and 12.6 for sulfadiazine, 57.1 and 12.6 for sulfamerazine, and 11.3 and 50.9 for sulfathiazole, respectively. The resulting recovery data for both sulfathiazole and the quantity c_t are shown in Table II. These results agree well with the known amounts of substances added.

The average recoveries found for sulfathiazole and the c_t were 100.2 ± 1.1 and 99.6 ± 0.6%, respectively. A 1.2–11.0-fold excess of the mixed pyrimidyl sulfonamides did not interfere with the sulfathiazole determination. Likewise, sulfathiazole did not interfere with the determination of the total pyrimidyl sulfonamide content. In addition, the sum of the

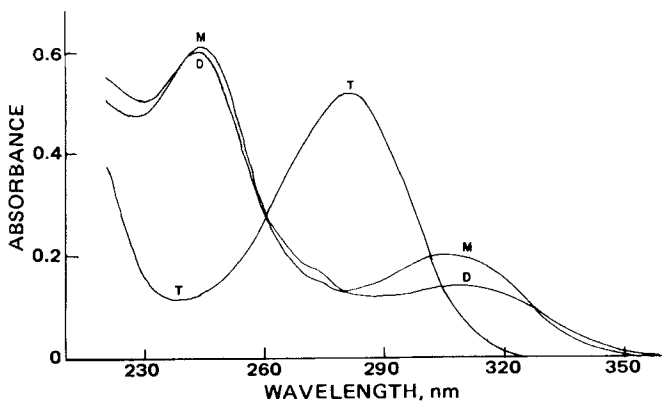


Figure 1—*UV spectra of sulfamerazine (curve M), sulfadiazine (curve D), and sulfathiazole (curve T) in 0.1* M *HCl. Concentration is 10.0 μg/ml.*

[4] Defined as the absorbance divided by the concentration expressed in grams per liter.

Table II—Recoveries from Synthetic Mixtures

Sample	Sulfathiazole μg/ml	Recovery, %	c_t[a] μg/ml	Recovery, %
1	3.94	98.5	5.93	98.8
2	2.98	99.3	6.94	99.2
3	2.99	99.7	6.96	99.4
4	4.02	100.5	6.03	100.5
5	4.02	100.5	6.03	100.5
6	4.00	100.0	6.03	100.5
7	2.01	100.5	8.02	100.3
8	1.00	100.0	8.84	98.2
9	2.97	99.0	7.98	99.8
10	2.99	99.7	7.96	99.5
11	5.01	100.2	5.96	99.3
12	3.06	102.0	8.00	100.0
13	1.00	100.0	9.94	99.4
14	4.07	101.8	7.98	99.8
15	2.03	101.5	9.97	99.7
16	4.98	99.6	6.94	99.2
17	4.98	99.6	6.94	99.2
18	4.96	99.2	6.95	99.3
19	1.03	103.0	10.90	99.1
Average		100.2 ± 1.1		99.6 ± 0.6

[a] c_t = sum of sulfamerazine and sulfadiazine concentration.

sulfathiazole content and the quantity c_t from Table II agreed well with the total sulfonamide concentrations given in Table I. The average recovery for the latter based on all 19 determinations was 99.7 ± 0.5%.

The effect of various excipients on the recovery from synthetic tablet mixtures also was investigated. The following excipients did not interfere when added individually or in combination to an equal weight of the mixed sulfonamides: starch, talc, magnesium stearate, microcrystalline cellulose, lactose, sodium lauryl sulfate, and dibasic calcium phosphate. The average recoveries for sulfathiazole and the c_t in the presence of these excipients were 99.8 ± 1.0 and 100.0 ± 0.8%, respectively.

Table III shows the results obtained from an analysis of an experimental triple sulfa tablet preparation. Five individual determinations were made from a single composite, and the resulting assays were in good agreement with the claimed levels.

The excellent recovery data reported with this procedure result from absorbance measurements taken at or near plateau regions in the spectrum. This fact is evident in Fig. 1 where the slope $\Delta A/\Delta\lambda$ is essentially constant at the wavelengths of interest. The first isoabsorptive wavelength (λ_1) is near the absorbance maximum of both pyrimidyl sulfonamides centered at 243 nm; the second isoabsorptive wavelength (λ_2) is located at the minimum between the first and second absorption bands of these same compounds. In addition, λ_1 and λ_2 occur at the minimum and maximum absorbances of sulfathiazole. Consequently, at these analytical wavelengths, the values of the absorptivity ratios a'_{iso}/a_3' and a''_{iso}/a_3'' are 5.05 (max) and 0.25 (min), respectively. These conditions are most favorable for the spectrophotometric determination of two components based on absorbance measurements at two selected wavelengths.

The location and intensity of the absorption bands in the UV absorbance spectra of I–III are both dependent on the solvent pH. Two additional pH's were examined to determine if the corresponding spectra were amenable to analysis by Eqs. 4 and 5. Figures 2 and 3 show the resulting spectra obtained in pH 6 acetate buffer and 0.1 *M* NaOH. Dual isoabsorptive points were found for the pyrimidyl compounds and showed a bathochromic displacement with increasing pH. For example, λ_1 shifted from 239 nm in acid to 249 nm in pH 6 buffer and to 266 nm in 0.1 *M*

Table III—Assay Results on an Experimental Triple Sulfa Tablet Composite[a]

Determination	Assay Results, % of Claim Sulfathiazole	Sulfamerazine plus Sulfadiazine
1	98.4	99.1
2	98.4	98.7
3	98.9	98.7
4	98.4	98.4
5	98.9	98.7
Average	98.6 ± 0.3	98.7 ± 0.3

[a] Claimed milligrams per tablet are: sulfathiazole, 186; and sulfamerazine plus sulfadiazine, 317.

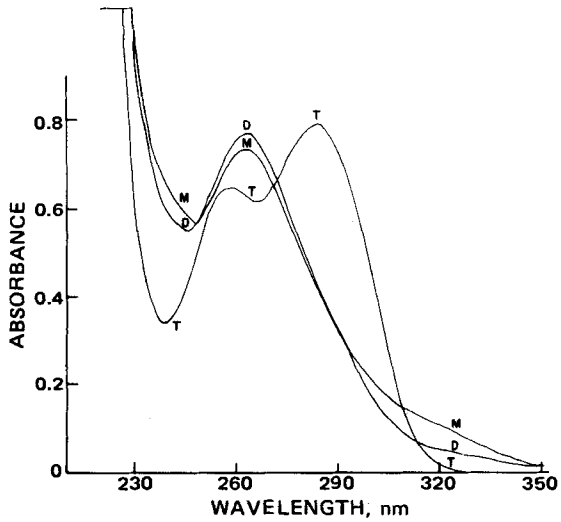


Figure 2—*UV spectra of sulfamerazine (curve M), sulfadiazine (curve D), and sulfathiazole (curve T) in pH 6 acetate buffer. Concentration is 10.0 μg/ml.*

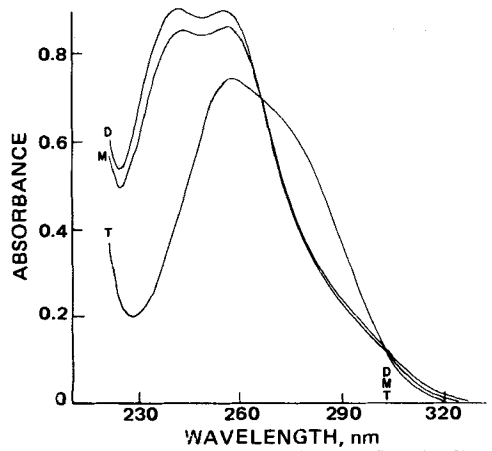


Figure 3—*UV spectra of sulfamerazine (curve M), sulfadiazine (curve D), and sulfathiazole (curve T) in 0.1 M NaOH. Concentration is 10.0 μg/ml.*

NaOH. Similarly, the shift observed for λ_2 was from 279 to 291 nm at the intermediate pH and finally to 303 nm in base.

Figures 2 and 3 show that the isoabsorptive points all occur on the steep slope of an absorption band. Also, the absorptivity ratios (a_{iso}/a_3), with one exception, are near unity. Consequently, absorbance measurements at these particular wavelengths in either solvent system are prone to considerable error and are not suitable for precise quantitative spectrophotometric analysis. This fact was demonstrated in a recovery study where both systems were applied to a synthetic tablet mixture. Based on six individual determinations, the best relative standard deviation obtained was only 6%. The use of 0.1 *M* HCl offers the obvious advantage of providing better precision.

The proposed method should be well suited for the routine analysis of a large number of samples. Both content uniformity testing and dissolution rate determinations of triple sulfa tablets would be practical examples.

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[x] To whom inquiries should be directed.