Colorimetric Determination of Some Sulfonamides with 9-Chloroacridine

J. T. STEWART, A. B. RAY, and W. B. FACKLER

Abstract □ A colorimetric procedure based on the reaction between 9-chloroacridine and primary aromatic amines has been applied to some sulfonamides and sulfonamide mixtures. It has been found to be comparable in sensitivity to other sulfonamide determinations, particularly the popular diazotization-coupling procedures. Quantitative data from several systems reveal that use of this procedure permits the determination of sulfonamides in the presence of an azo dye, tetracycline hydrochloride, sodium penicillin G, hexamethyleneamine, and other sulfonamides. Comparative analyses were performed with the method of Bratton and Marshall on sulfamerazine, sulfamethoxypyridazine, and succinylsulfathiazole tablets.

Keyphrases □ Sulfonamides, sulfonamide mixtures—analysis □ 9-Chloroacridine—chromogenic reagent □ Colorimetric analysis—spectrophotometer

The objective of this investigation was to evaluate a colorimetric method of analysis for sulfonamides based on the reaction between organic amines and 9-chloroacridines to yield highly-colored aminoacridine hydrochlorides. The assay procedure applied to primary aromatic amines has been reported previously by this laboratory (1).

In this paper, the method has been applied to the analysis of several sulfonamides and sulfonamide mixtures. A comparative study of this technique was made with the procedure of Bratton and Marshall (2).

EXPERIMENTAL

Apparatus—Spectra and absorbance measurements were made with spectrophotometers.[1, 2] Matched cells with a 1-cm. optical path were used.

Reagents and Chemicals—9-Chloroacridine[3] was used as the chromogenic reagent. Powdered samples of sulfamethizole,[4] sulfisoxazole,[5] sulfamethoxypyridazine,[6] succinylsulfathiazole,[7] sulfamerazine,[8] and sulfathiazole[9] were used in the analytical procedure for preparation of standard curves. Sulfamethoxypyridazine,[10] succinylsulfathiazole,[11] and sulfamerazine[12] tablets were used in the comparative study with the Bratton-Marshall procedure. 2,6-Diamino-3-phenylazopyridine hydrochloride,[13] sodium penicillin G,[14] tetracycline hydrochloride,[15] and hexamethyleneamine USP[16] were also used in the analysis. All other chemicals used were the highest grade of the commercially available materials.

Solutions (10^{-6} mole ml.$^{-1}$) were prepared by dissolving weighed amounts of the sulfonamides and hexamethyleneamine in ethanol and weighed amounts of 2,6-diamino-3-phenylazopyridine hydrochloride, sodium penicillin G, and tetracycline hydrochloride in water. Solutions of 9-chloroacridine (10^{-12} mole ml.$^{-1}$) were prepared immediately before use by dissolving weighed amounts in ethanol.

Procedure—One milliliter of an ethanolic solution of sulfa drug (10^{-6} mole ml.$^{-1}$) was placed in a 10-ml. volumetric flask. The pH of the drug solution was adjusted to pH 6–7 by the addition of either 10% v/v hydrochloric acid or sodium hydroxide solution. To this was added 2 ml. of an ethanolic solution of 9-chloroacridine (10^{-12} mole ml.$^{-1}$). Then the pH was adjusted to approximately 4 with 10% v/v aqueous hydrochloric acid. The solution was shaken and allowed to sit for 5 min. at room temperature followed by the addition of ethanol to volume and absorbance measured at 435 mμ. Absorbance measurements were corrected for reagent blanks in the procedure.

RESULTS AND DISCUSSION

The results of this investigation indicate that the reaction between sulfonamides containing a primary aromatic amino group and 9-chloroacridine to yield highly-colored aminoacridine hydrochlorides can be utilized as a suitable assay procedure for sulfonamides. The absorption curve in the visible spectrum for a typical sample of sulfisoxazole shows an absorption maximum at 435 mμ. Reagent blank readings at this wavelength are very low.

In comparing absorption curves of the colored solutions obtained with equimolar concentrations of the various sulfonamides containing primary aromatic amino groups, it was noted that the curves were almost identical. Compounds such as sulfamethizole, sulfamethoxypyridazine, sulfamerazine, and sulfathiazole all produce color which absorbs at the same wavelength maximum and with essentially the same intensity as does sulfisoxazole. Structurally all of these compounds have a primary aromatic amino group in the position *para* to the sulfonamido linkage.

Succinylsulfathiazole gives no color formation with this procedure. However, saponification with base yields sulfathiazole, which does react with 9-chloroacridine to produce the desired color for the analytical procedure.

Standard curves can be prepared by plotting observed absorbance readings *versus* the volumes taken of equimolar concentrations of various sulfonamides. In all cases, Beer's law holds for this system.

Quantitative data from several systems shown in Table I reveal that use of this procedure permits the determination of sulfon-

Table I—Analysis of Known Sulfonamide Mixtures for Sulfonamide

Mixture	Components, Concn. of 2.500 × 10^{-8} mole ml.$^{-1}$	Sulfonamide—Found, mole ml.$^{-1}$ × 10^{-8}	Sulfonamide—% of Theory
1	Sulfamethizole Hexamethyleneamine	2.500	100.0
2	Sulfamethizole 2,6-Diamino-3-phenylazopyridine hydrochloride Tetracycline hydrochloride	2.480	99.2
3	Sulfamethoxypyridazine 2,6-Diamino-3-phenylazopyridine hydrochloride	2.500	100.0
4[a]	Sulfisoxazole Succinylsulfathiazole	2.500	100.0
5	Sulfisoxazole Sodium penicillin G	2.490	99.6

[a] Mixture analyzed for sulfisoxazole content.

[1] Perkin-Elmer, model 202.
[2] Beckman, model DU.
[3] Eastman Organic Chemicals.
[4] Ayerst Laboratories, New York.
[5] Hoffmann-LaRoche, Inc., Nutley, N. J.
[6] Parke-Davis and Co., Detroit, Mich.
[7] Merck Sharp and Dohme, West Point, Pa.
[8] American Pharmaceutical Company, New York.
[9] City Chemical Corporation, New York.
[10] Marketed as Midicel by Parke-Davis and Co.
[11] Marketed as Sulfasuxidine by Merck Sharp and Dohme.
[12] Marketed as Sulfamerazin N. F. by Eli Lilly and Co.
[13] K and K Laboratories, New York.
[14] Eli Lilly and Co., Indianapolis, Ind.
[15] Chas. Pfizer and Co., Inc., New York.
[16] Fisher Scientific Co.

Table II—Determination of Sulfamerazine, Sulfamethoxypyridazine, and Succinylsulfathiazole in Tablets by the 9-Chloroacridine Method and the Method of Bratton and Marshall

	9-Chloroacridine Method		Bratton-Marshall Method	
	Mean % of Labeled Amount	*SD* of Mean, %	Mean % of Labeled Amount	*SD* of Mean, %
Sulfamerazine tablets	99.00	0.45	98.75	0.25
Sulfamethoxypyridazine tablets	99.52	0.27	100.63	0.55
Succinylsulfathiazole tablets	99.43	0.40	99.20	0.46

amides containing a primary aromatic amino group in the presence of other sulfa derivatives, such as succinylsulfathiazole, and in the presence of 2,6-diamino-3-phenylazopyridine hydrochloride, tetracycline hydrochloride, sodium penicillin G, and hexamethyleneamine. The latter compounds are found in various combinations with sulfonamides in commercially available dosage forms. It was shown from earlier studies that primary, secondary, and tertiary aliphatic amines, secondary and tertiary aromatic amines, heterocycles, and carbonyl-containing compounds also do not interfere with this method (1).

The analytical method is essentially a micro procedure, and sensitivity is in the range of 10^{-7}–10^{-8} mole ml.$^{-1}$ of sulfonamide, which makes it comparable to other sulfa determinations, particularly the popular diazotization-coupling procedures.

A favorable characteristic of the analysis is that the absorbance of the product formed is stable and does not fade over a 24-hr. period. This is an advantage over the colorimetric method of Bratton and Marshall. In the latter method, absorbance readings must be made within 15 min. after color development, due to precipitation of the azo dyes in the method (3). The 9-chloroacridine method does not involve diazotization. Thus, it eliminates the need for freshly prepared sodium nitrite and ammonium sulfamate solutions required with the Bratton-Marshall technique. Control of pH is required in both methods.

The method of analysis for sulfonamides by the 9-chloroacridine approach was carried out for various sulfonamides, and comparative analyses were performed using the colorimetric procedure of Bratton and Marshall. Assays were performed on sulfamerazine, sulfamethoxypyridazine, and succinylsulfathiazole in tablets. With succinylsulfathiazole, saponification with sodium hydroxide as outlined in NF XI was required to form the primary amine (4). The commercially available sulfamethoxypyridazine tablets used were colored with a yellow dye; but it was found that for the dilutions used, the absorbance from the color was not sufficient to interfere with the assays by either method.

The procedure outlined by Connors was used for the analysis by the Bratton-Marshall method (5).

Four determinations by each method were performed for each sulfonamide. The mean percent of labeled amount and the percent standard deviation of the mean for each sulfonamide are shown in Table II for both methods (6).

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 16, 1969 from the *Department of Medicinal Chemistry, University of Georgia School of Pharmacy, Athens, GA 30601*

Accepted for publication July 10, 1969.

Presented to the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

Supported in part by the Office of General Research, University of Georgia.

Inclusion Compounds in Pharmaceutical Analysis I: Determination of Dienestrol in Dienestrol Cream

BARBARA J. FORMAN and LEE T. GRADY

Abstract □ Large ratios of monostearin to dienestrol in cream formulations complicate the development of an assay for dienestrol. Monostearin was removed easily in a test-tube procedure by channel-type inclusion in urea. Dienestrol recovery was complete and reproducible analyses were obtained by polarography of the nitrosophenol derivative. Urea inclusion may offer a general approach to analytical methodology where long-chain compounds need be separated from active ingredients.

Keyphrases □ Dienestrol creams—analysis □ Monostearin removal, dienestrol creams—urea inclusion compound □ Column chromatography—separation □ Polarography, organic—analysis

Isolation of dienestrol and related compounds from formulations containing surfactants or hydrocarbons has required troublesome steps such as column chromatography. Gottlieb (1) separated diethylstilbestrol from creams and ointments using toluene at reflux to break emulsions and subsequently isolated the drug on an alumina column. Nevertheless, he reported poor recovery in the presence of monostearin. A more recent illustration (2) was the determination of diethylstilbestrol in a water-dispersibile suppository using an alumina column step prior to quantitative TLC.

In developing an assay for dienestrol in dienestrol cream,[1] the authors separated the drug from at least a thousand-fold excess of monostearin. An existing polarographic method was used for the determinative step. Monostearin interfered with this step by causing gross distortion of the polarograms.

[1] To be official in NF XIII.