ACKNOWLEDGMENTS AND ADDRESSES

Received August 25, 1972, from the *School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO 64110 and † Marion Laboratories, Inc., Kansas City, MO 64147

Accepted for publication December 19, 1972.

Presented to the 19th Canadian Conference on Pharmaceutical Research, Edmonton, Alberta, August 1972.

The authors thank Marion Laboratories, Inc., for financial support of this project.

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Analysis of Trisulfapyrimidines by High-Pressure Liquid Chromatography

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Abstract □ The application of high-pressure liquid chromatography to the separation and analysis of trisulfapyrimidines in pharmaceutical dosage forms is demonstrated. The preparation of samples of both tablet and suspension dosage forms is simple and rapid. The chromatographic conditions chosen optimize the separation of sulfadiazine, sulfamerazine, and sulfamethazine and allow quantitative analysis of these trisulfapyrimidines in a reasonable time.

Keyphrases □ Trisulfapyrimidine formulations—analysis, high-pressure liquid chromatography □ Sulfa drugs, sulfadiazine-sulfamerazine-sulfamethazine—separation, analysis, high-pressure liquid chromatography □ High-pressure liquid chromatography—analysis, trisulfapyrimidine formulations

The separation and quantitative analysis of sulfadiazine, sulfamerazine, and sulfamethazine in pharmaceutical dosage forms present a difficult problem to the pharmaceutical analyst. Current methods and proposed modifications of them are slow and tedious. The USP method (1) uses a paper chromatographic separation prior to colorimetric determination, by means of the Bratton–Marshall reaction, of the eluted individual sulfonamides. Modifications proposed by Kunze and coworkers (2, 3) require extreme care for satisfactory results. Banes and Riggleman (4) recently proposed a hybrid assay for trisulfapyrimidine preparations in which total sulfonamides are measured colorimetrically by the Bratton–Marshall procedure. Sulfadiazine is then measured colorimetrically by means of its specific reaction with thiobarbituric acid; sulfamethazine is separated from its homologs by column partition chromatography and is then determined by UV spectrophotometry. The third sulfonamide is obtained by difference. A qualitative chromatogram is used to confirm that only the three sulfonamides are present.

The determination of trisulfapyrimidines in dosage forms by high-pressure liquid chromatography was first reported by Poet and Pu (5). A recent paper by Kram (6) reported the conditions for the separation of a number of sulfapyrimidines. The determination of trisulfapyrimidines by high-pressure liquid chromatography overcomes or circumvents many shortcomings of the previously reported methods. The preparation of samples is simple and rapid, and separation and analysis times are reasonably short.

EXPERIMENTAL[1]

Mobile Phase and Reagents—The mobile phase, 0.2 *M* disodium phosphate solution adjusted to pH 6.0 with 85% phosphoric acid, was prepared fresh daily. Sodium hydroxide and sulfuric acid solutions (1 *N*) were required.

Internal Standard Solution—The stock internal standard solution, 1200 mcg./ml., was prepared by first dissolving 120 mg. of sulfadimethoxine in 5 ml. of 1 *N* sodium hydroxide solution and then diluting to 100 ml. with distilled water.

Trisulfapyrimidine Stock Standard Solution—The trisulfapyrimidine stock standard solution was prepared by first dissolving 120 mg. each of sulfadiazine, sulfamerazine, and sulfamethazine in 5 ml. of 1 *N* sodium hydroxide solution and then diluting to 100 ml. with distilled water. This solution contained each of the trisulfapyrimidines at a concentration of 1200 mcg./ml.

Standard Curve Solutions—These solutions were prepared by suitable dilution of the stock standard solution with distilled water. They contained each of the trisulfapyrimidines at a concentration between 108 and 132 mcg./ml. and the internal standard at a concentration of 120 mcg./ml.

Preparation of Tablet Sample—For a single-tablet analysis, the weight of a single tablet was determined. For a batch analysis, the weight of a pool of several tablets was determined and the average tablet weight was calculated. The sample was ground to a fine powder. An accurately weighed portion of the powdered sample, equivalent to 36 mg. of total trisulfapyrimidines, was transferred to a 100-ml. volumetric flask containing 10 ml. of 1 *N* sodium hydroxide solution. The stoppered flask was shaken on a mechanical reciprocal shaker for 15 min. A total of 9 ml. of 1 *N* sulfuric acid was added while the flask contents were swirled. Some distilled water was added, followed by 10 ml. of internal standard solution and enough additional distilled water to make 100 ml. of solution. A portion of the well-shaken extract, transferred to a glass-stoppered test tube, was centrifuged for 10 min. at 2000 r.p.m., and the supernate was used for analysis.

Preparation of Suspension Sample—A weight of well-shaken trisulfapyrimidine suspension, equivalent to 36 mg. of total trisulfapyrimidines, was transferred to a 100-ml. volumetric flask, and 10 ml. of 1 *N* sodium hydroxide solution was added. Preparation of the sample was continued as described earlier for tablet samples.

Conditions for Chromatographic Separation—The important features of the liquid chromatograph used were described in detail elsewhere (7, 8). The degassed mobile phase was passed through the cation-exchange column under a pressure of 1000 psig., to obtain a flow rate of 0.7–0.8 ml./min. at room temperature, until a stable

[1] A DuPont liquid chromatograph (model 820) equipped with a UV monitor, an Infotronics integrator (Model 10-AB-2), with digital printout, and a DuPont packed "Zipax" SCX cation-exchange column, 1 m. long, 6.35-mm. (0.25-in.) o.d. and 2.1-mm. i.d., was used. The column contained approximately 6 g. of "Zipax" support, having about a 1% loading of the cation-exchange polymer.

Table I—Effect of the Mobile Phase Molarity on Retention Time[a]

Compound	0.01 *M*	0.1 *M*	0.2 *M*
Sulfadiazine	64	67	70
Sulfamerazine	97	103	112
Sulfadimethoxine	140	155	182
Sulfamethazine	240	270	308

[a] Expressed as the number of seconds elapsed between injection and attainment of the chromatographic peak maximum.

baseline was obtained at a photometer attenuation of 8×10^{-3}. Replicate 4-μl. injections of standard and sample solutions were made, using a 10-μl. syringe[2]. The chart recorder provided a record of the elution of the sulfonamides from the column as peaks on a chromatogram, while the electronically calculated peak areas were printed out on paper tape.

RESULTS AND DISCUSSION

The purpose of this study was to determine the operating conditions for chromatography that would optimize the resolution of the trisulfapyrimidines in a reasonable time with a suitable level of precision.

The use of an internal standard was intended to minimize the effects of minor procedural variations. Sulfadimethoxine was chosen as the internal standard because it was eluted between two existing peaks in the chromatographic system employed and, in solution, demonstrated properties similar to those of the other trisulfapyrimidines.

The operating conditions studied included pH and molarity of the mobile phase, as well as pressure and flow rate. The effect of pH of the mobile phase on the separation of the trisulfapyrimidines is illustrated in Fig. 1. Although no separation of sulfamerazine and sulfadimethoxine was obtained at pH 5.7, adequate separation was obtained at pH 6.0. The compounds were well separated at pH 6.3; however, the higher the pH, the longer was the time required for elution of the more strongly retained sulfonamides. Therefore, pH 6.0 was chosen for the separation to minimize the time required. The effect of the molarity of the mobile phase on the time that a sulfonamide is retained on the column is shown in Table I. These data were obtained in a preliminary screening study using a faster mobile phase flow rate than was finally chosen. Despite an unexpected moderate increase in the retention time for the two more strongly retained sulfonamides, a 0.2 *M* disodium phosphate buffer solution, pH 6.0, was selected for the mobile phase to ensure the buffering stability of the system. Because buffer solutions that had been stored or used over extended periods of time adversely affected the chromatogram baseline, a fresh supply of buffer solution was prepared daily. The chosen pressure of 1000 psig., which produced a flow rate of 0.7–0.8 ml./min., resulted in separation of the sample components within 15–20 min.

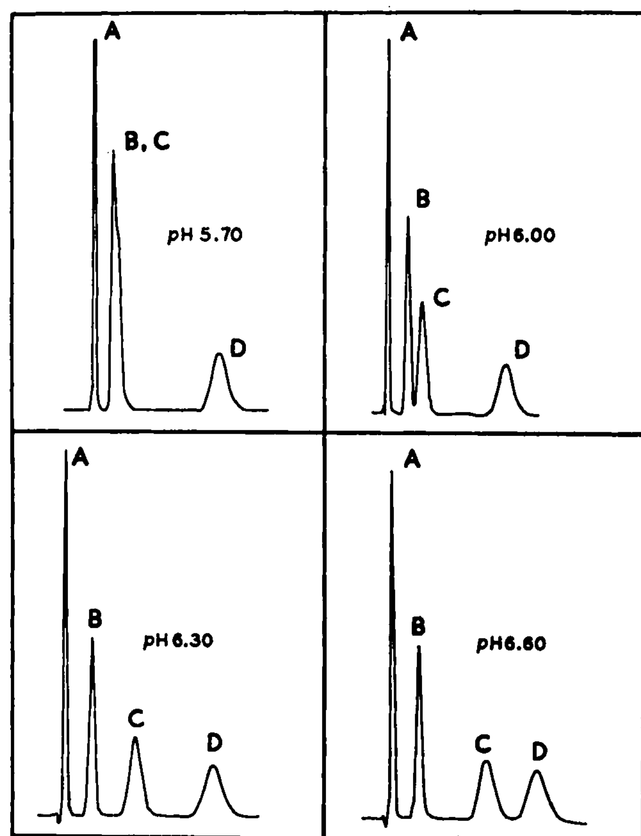

Figure 1—*Effect of pH on the separation of sulfadiazine (A), sulfamerazine (B), sulfadimethoxine (C), and sulfamethazine (D).*

Table II—Response Factors for Standard Solutions[a]

Sulfonamide	Average Response Factor	Standard Deviation	Variance	Coefficient of Variation, %
Sulfadiazine	1.2827	±0.0332	0.0011	2.6
Sulfamerazine	1.2356	±0.0246	0.0006	2.0
Sulfamethazine	0.9162	±0.0221	0.0005	2.4

[a] These data represent four injections of each of five standard solutions.

Table III—Concentrations of Trisulfapyrimidines in Sulfonamide Tablets and Suspensions

Sample	Sulfadiazine mg.	Sulfadiazine %	Sulfamerazine mg.	Sulfamerazine %	Sulfamethazine mg.	Sulfamethazine %
Tablet A[a]	161.7	96.8	164.9	98.7	163.0	97.6
Tablet B	167.2	100.1	167.8	100.5	172.4	103.2
Tablet C	165.6	99.2	162.0	97.0	164.7	98.6
Tablet D	161.3	96.6	166.9	99.9	169.7	101.6
Suspension A[b]	165.9	99.3	158.7	95.0	158.9	95.2
Suspension B	180.4	108.0	169.1	101.3	171.8	102.9

[a] Theory = 167.0 mg. of each sulfonamide per tablet. [b] Theory = 167.0 mg. of each sulfonamide/5 ml. of suspension formulation. In these calculations, the specific gravity for the particular suspension, previously determined, was employed.

The area under the curve for each peak on the chromatograms was calculated electronically. The response factor, the ratio of each sulfonamide peak area to the area of the internal standard, was calculated for each chromatogram. Because the response factors were linearly related to sulfonamide concentration, average response factors for a 120-mcg./ml. concentration of each sulfonamide were computed from the ratios (Table II). Response factors for each sulfonamide were determined for a limited number of standard solutions on the same day that samples were assayed. Thus, the effect of changes in response factors due to possible changes in the operating conditions or in the column could be minimized.

Analytical data obtained for four representative lots of tablet formulations and two suspension formulations are presented in Table III. The calculated coefficients of variation for replicate sample injections ranged from 0.9 to 4.0%. The data in Table III demonstrate the utility of high-pressure liquid chromatography for the analysis of these trisulfapyrimidines in pharmaceutical dosage forms.

[2] Hamilton-701N.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 17, 1972, from the *Squibb Institute for Medical Research, New Brunswick, NJ 08903*
Accepted for publication December 14, 1972.
The authors express their appreciation to Dr. G. Brewer and Dr. K. Florey for their encouragement and suggestions during this study, and to Dr. D. Frost for his helpful discussions during the preparation of this manuscript.
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PHARMACEUTICAL TECHNOLOGY

Evaluation of Inhalation Aerosols Using a Simulated Lung Apparatus

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Abstract ☐ A model lung chamber was designed for the evaluation of oral inhalation aerosols. The lung chamber was a compartmentalized unit based on certain parameters of the human respiratory tract. A vacuum system was used to regulate the flow rate through the chamber. Based on studies of air flow rate and evaluations with medicinal aerosol units, a vacuum of 30.4 cm. (12 in.) of mercury was chosen as the most suitable pressure for analysis of the aerosol samples within the chamber. Sampling of the chamber was by gravity, deposition of the nebula in sample collection vials attached to the base of each compartment, or full rinsing of each compartment. Samples were analyzed spectrophotometrically. The particle-size distributions of aerosolized talc samples from each chamber compartment were determined to evaluate the separation characteristics of the model lung chamber. Solutions of varying strengths of isoproterenol hydrochloride and phenylephrine hydrochloride aerosolized using several common aerosol devices demonstrated the model lung chamber to be a suitable device for evaluating medicinal and pharmaceutical aerosol units.

Keyphrases ☐ Inhalation aerosols—evaluated using simulated lung chamber ☐ Aerosols, inhalation—evaluated using simulated lung chamber ☐ Simulated lung chamber—design used to evaluate inhalation aerosols ☐ Lung chamber, simulated—design used to evaluate inhalation aerosols

Until recently, aerosol inhalation therapy has received comparatively little attention when compared with the more conventional dosage routes for drugs. However, with the development of pressurized aerosol technology and portable aerosol-generating equipment and an increasing appreciation of inhalation as a route for the administration of medicinals, considerable interest has developed in this area.

Inhalation therapy may be used to administer drugs for local and/or systemic response. By effecting depth of penetration and retention of inhaled medicinals, it is possible to obtain a purely local action without systemic effects or a combination of local and systemic action (1–3). Several parameters influence the penetration and deposition of inhaled materials in the lung.

Extensive research (4–12) has been conducted on the relationship of particle size to distribution and retention in the lungs. The conflict of theories and experimental results from these investigators has been attributed to such variable factors as species of animal used, nonuniform breathing rates, methods of measurement of particle-size distribution, methods of administering the agents used, and effects of lung moisture content on the size of inhaled particles (3, 6, 7, 13, 14). All authors agree, however, that depth of penetration increases with decreasing particle size while whole lung retention increases with increasing particle size (1, 6, 15, 16). The optimum particle-size range for inhalation of medicinals into the lungs is currently accepted as 0.5–5.0 μ (17, 18).

While the importance of particle size in inhalation therapy has been well documented, the other parameters effecting deposition and retention of inhaled matter have received less attention. In addition, pharmaceuticals are available in pressurized form for administration as either liquid droplets or fine solid particles. However, no evidence in the literature documents the superiority of solution systems of inhalation aerosols over suspension systems or vice versa.

Since *in vivo* evaluation of inhaled materials can lead to a disparity of results due to variable factors, there is a need for a good *in vitro* method of evaluating inhaled materials. This study describes the development of a collection chamber for *in vitro* analysis of materials from pressurized pharmaceutical aerosols or other aerosol-generating equipment.