Stability-Indicating Sulfa Drug Analysis Using High-Performance Liquid Chromatography

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Abstract □ Sensitive and efficient methods for sulfonamide determination as single entities and in combination with other drug substances in pharmaceutical dosage formulations were developed using high-performance liquid chromatography. These stability-indicating procedures involved a nitrile bonded phase column and nonaqueous mobile phases having diverse polarities. Sample potency was determined using peak height measurements. The methods may be used to determine trace sulfonamide quantities because detection limits are in the nanogram range.

Keyphrases □ High-performance liquid chromatography—analysis, sulfonamides in various pharmaceutical formulations, stability indicating □ Sulfonamides, various—analysis, high-performance liquid chromatography, various pharmaceutical formulations, stability indicating □ Dosage forms, various—analysis, sulfonamides, high-performance liquid chromatography, stability indicating

Methods for sulfonamide determination include potentiometric (1) or nonaqueous (2) titrations as well as direct spectrophotometric measurement (3). When sulfonamides are formulated in combination with other drug substances, the analysis of all components often requires more than one procedure, none being stability indicating. An interest in determining the active constituents of multicomponent sulfa drugs utilizing a single analytical method led to the development of a high-performance liquid chromatographic (HPLC) procedure.

BACKGROUND

Several studies were published on sulfonamide separation and determination using HPLC. Sulfonamides varying widely in pKa and hydrophobicity were separated using ion-pair partition chromatography (4). Liquid chromatographic sulfonamide separation also was accomplished using an "ether" bonded phase packing (5). Sulfonamides, including sulfisoxazole, sulfamethoxazole, *N'*-acetylsulfisoxazole, and sulfisoxazole diolamine, were separated on a strong anion-exchange column using a 0.01 *M* sodium borate mobile phase containing varying amounts of sodium nitrate (6).

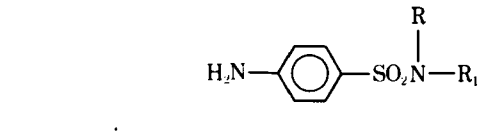
Retention volume data for various sulfonamides on a pellicular-bonded reversed-phase packing, a pellicular packing coated with squalene, or a pellicular packing coated with octanol were reported (7). The partition coefficients, pKa values, and biological activity of each compound were correlated with retention volume.

Trace level quantitation of sulfamethazine residue in bovine tissue was reported (8). With this method, 0.07 ppm of sulfamethazine in tissue could be detected.

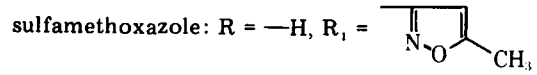
This paper describes a sensitive and efficient HPLC procedure for sulfonamide determination in pharmaceutical formulations. The assays involve a nitrile bonded phase column and nonaqueous mobile phases of varying polarity. The assays are stability indicating because precursors and decomposition products can be determined simultaneously with the drugs. Furthermore, compounds present in combination drug formulations are quantitated.

EXPERIMENTAL

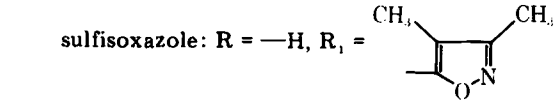
Apparatus—A high-performance liquid chromatograph equipped with a UV detector[1] was used to monitor the eluted compounds at 254 nm. Separation was obtained on an alkylnitrile bonded phase column[2].



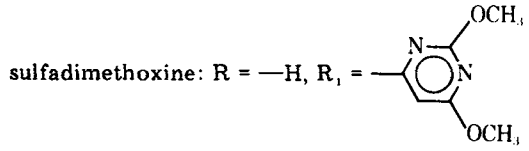
sulfanilamide: R = —H, R_1 = —H



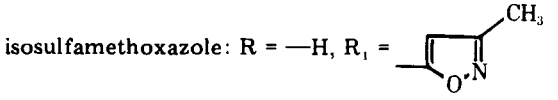
sulfamethoxazole: R = —H, R_1 =



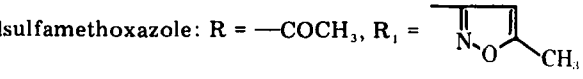
sulfisoxazole: R = —H, R_1 =



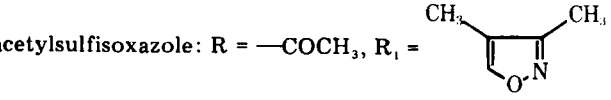
sulfadimethoxine: R = —H, R_1 =



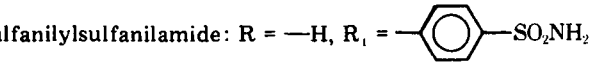
isosulfamethoxazole: R = —H, R_1 =



acetylsulfamethoxazole: R = —$COCH_3$, R_1 =



acetylsulfisoxazole: R = —$COCH_3$, R_1 =



sulfanilylsulfanilamide: R = —H, R_1 =

Reagents and Chemicals—The methanol, methylene chloride, and ethyl acetate were ACS reagent grade. "Distilled-in-glass" heptane[3] was used. The diethylamine and hydrochloric acid were reagent grade. Released lots of sample material and in-house reference standards were used[4].

Mobile Phases—A mobile phase of methanol–methylene chloride–heptane (10:15:75) was used for sulfamethoxazole, *N'*-acetylsulfisoxazole, sulfisoxazole, and sulfadimethoxine analyses as well as for samples containing phenazopyridine hydrochloride in combination with sulfamethoxazole or sulfisoxazole.

A mobile phase of diethylamine–ethyl acetate–methanol–heptane (1:8:16:75) was used for samples containing trimethoprim or ormetoprim in combination with sulfamethoxazole or sulfadimethoxine, respectively.

Internal Standard Solution—Sulfanilylsulfanilamide was prepared by the Bauer (9) procedure and used as an internal standard. Approximately 600 mg of this material was accurately weighed into a 100-ml volumetric flask and diluted to volume with methanol (internal standard solution).

Sample Solution—Each sulfa drug solution was prepared by dissolving 100 mg in 100 ml of methanol except when in combination with

[1] Model 830, E. I. du Pont de Nemours & Co., Wilmington, Del.

[2] MicroPak CN-10 (25 cm × 2 mm i.d.), Varian Associates, Sunnyvale, Calif.

[3] Burdick & Jackson Laboratories, Muskegon, Mich.

[4] Hoffmann-La Roche, Nutley, N.J.

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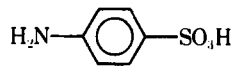
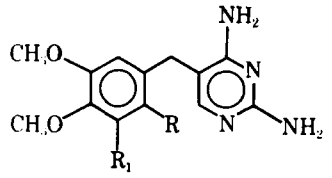
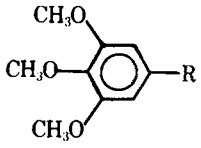


sulfanilic acid

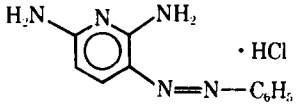


trimethoprim: R = —H, R_1 = —OCH_3
ormetoprim: R = —CH_3, R_1 = —H



trimethoprim
precursor A: R = —CH_2—CH(CN)—CH(OCH_3)(OCH_3)

trimethoprim
precursor B: R = —C≡C—CH_2OCH_3 (CN)



phenazopyridine hydrochloride

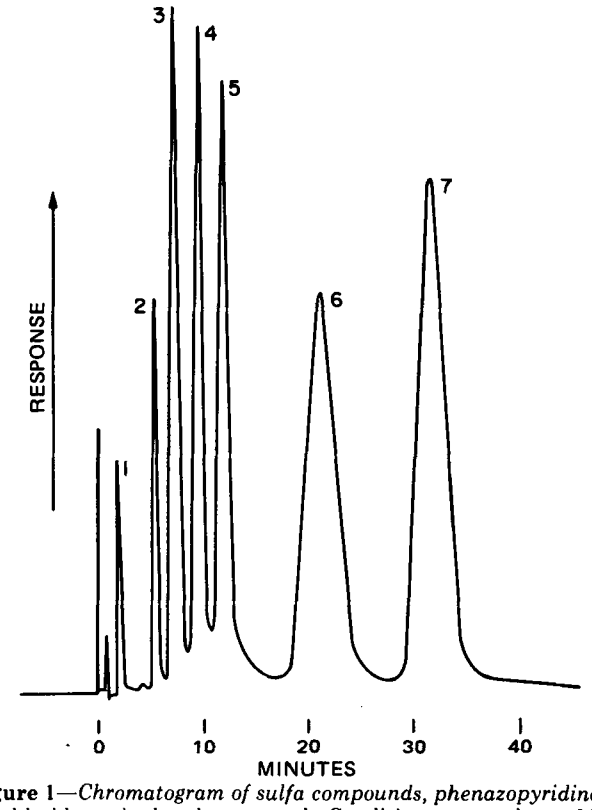


Figure 1—*Chromatogram of sulfa compounds, phenazopyridine hydrochloride, and related compounds. Conditions were: column, Micro-Pak CN-10; mobile phase, methanol–methylene chloride–heptane (10:15:75); and flow rate, 1.0 ml/min. Key: 1, phenazopyridine hydrochloride; 2, N′-acetylsulfamethoxazole; 3, sulfamethoxazole; 4, sulfisoxazole; 5, sulfanilamide; 6, isosulfamethoxazole; and 7, sulfanilylsulfanilamide.*

trimethoprim or ormetoprim, where 0.1 *N* HCl in methanol was used as the solvent. Four milliliters of the clear solution was pipetted into a 10-ml volumetric flask containing 2 ml of the internal standard solution and diluted to volume with methanol (working sample solution).

Reference Standard Solution—Reference standard solutions were prepared by diluting reference standard materials to a concentration similar to that of the samples with the same solvent (working reference standard solution).

Procedure—Five microliters of working sample and working reference standard solutions were alternately injected into the liquid chromatograph to determine the retention volumes for the compounds. Duplicate standards and samples were chromatographed, and the respective responses were determined using peak height measurements.

Calculations—Relative response ratios (*RR*) were calculated for the working reference standard solution using the chromatographic data. Calculation of drug potency for each tablet was made using:

$$\text{mg of drug/tablet} = \frac{(H_{spl})(W_{int})(\text{A.W.T.})(0.5)}{(H_{int})(W_{spl})(RR)} \qquad \text{(Eq. 1)}$$

where:

H_{spl} = sample peak height
H_{int} = internal standard in sample solution peak height
W_{int} = internal standard weight (milligrams)
W_{spl} = sample weight (milligrams)
RR = response ratio
0.5 = dilution factor
A.W.T. = average tablet weight (milligrams)

Formulas for calculating potency for other dosage forms (suspensions, solutions, creams, *etc.*) were adjusted to fit the particular dosage form.

RESULTS AND DISCUSSION

The sulfa drugs sulfamethoxazole, sulfisoxazole, *N′*-acetylsulfisoxazole, and sulfadimethoxine, both as single entities and in combination with ormetoprim, trimethoprim, and phenazopyridine hydrochloride, were analyzed successfully using normal phase partition HPLC. Two different mobile phases and a nitrile-bonded phase column were used to separate and determine all compounds of interest within a reasonable time. Typical chromatograms are shown in Figs. 1 and 2.

While the sulfa drugs could be chromatographed using either mobile phase, trimethoprim and ormetoprim did not elute in a reasonable time in the mobile phase without diethylamine. Sulfanilamide and sulfanilic acid, which are sulfonamide degradation products, were well resolved

Table I—Comparison of HPLC with Wet Chemical Results

Dosage Form	Compound Determined	Results for Various Lots, % claim HPLC	Wet Chemical
Tablet	Sulfisoxazole	100.1	102.1
		100.6	102.3
	Sulfamethoxazole	99.1	103.6
		100.6	101.6
	Sulfadimethoxine	100.8	104.4
		101.9	105.6
	Phenazopyridine hydrochloride	103.2	100.6
		100.0	100.0
	Trimethoprim	98.5	101.1
		101.3	99.9
	Ormetoprim	103.0	100.0
		105.0	102.0
Suspension	Acetylsulfisoxazole	99.7	102.0
		100.8	102.0
	Sulfamethoxazole	99.7	104.0
		98.0	101.6
	Trimethoprim	102.8	104.5
Cream	Sulfisoxazole	100.4	99.0
		99.3	98.0
Solution	Sulfisoxazole	105.5	106.0
		102.0	102.8

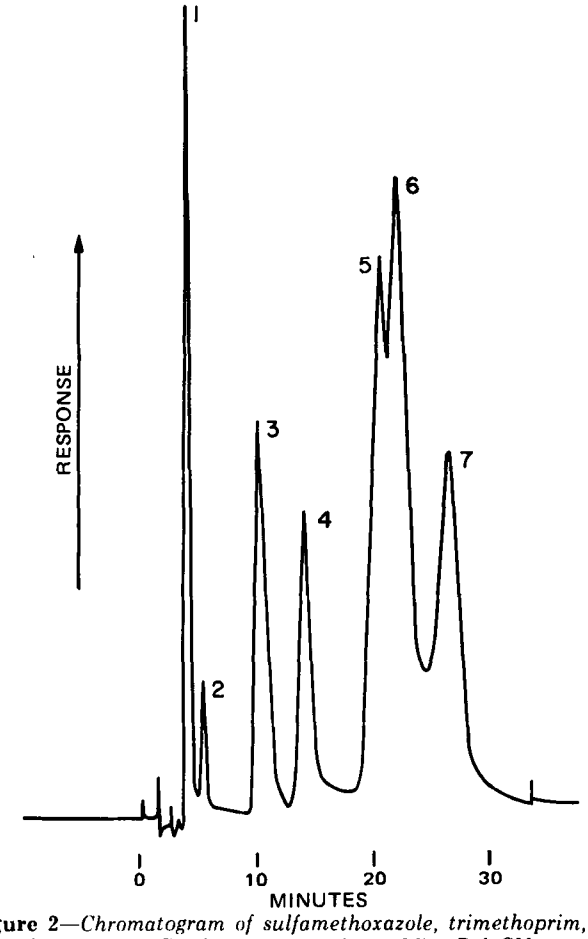


Figure 2—*Chromatogram of sulfamethoxazole, trimethoprim, and related compounds. Conditions were: column, MicroPak CN-10; mobile phase, methanol–ethyl acetate–diethylamine–heptane (16.8:1:75); and flow rate, 1.0 ml/min. Key: 1, trimethoprim precursor A; 2, trimethoprim precursor B; 3, trimethoprim; 4, N'-acetylsulfamethoxazole; 5, sulfanilamide; 6, sulfamethoxazole; and 7, isosulfamethoxazole.*

from the compounds of interest using the system without diethylamine; sulfanilic acid was eluted after all of the other sulfa compounds.

In some instances, 0.1 *N* HCl in methanol was chosen as the extraction solvent to ensure complete extraction of the drug substances from the matrix. Comparison of the HPLC results with results obtained using wet chemical procedures is made in Table I. Peak height responses *versus* amounts injected were linear for the formulation ranges for all components; however, deviations from linearity were found for many constituents when very high amounts were injected. Using the procedures described in this report, detection limits in the nanogram range were obtained. The determination of any compound tested in Table I, alone or in combination with another active drug substance, had a relative standard deviation of ±1.2%. An exception was found for the determination of trimethoprim and sulfamethoxazole formulated together, where the relative standard deviation was ±2.5%.

To ensure column-to-column duplication of the separation, three columns from the same supplier were evaluated. Differences in component retention times were noted with these columns, but only a slight modification in the mobile phase ratio was needed to effect resolution within a reasonable time.

In summary, the HPLC method presented was reliable, reproducible, rapid, and specific and should be useful for sulfa drug determinations in tablets, solutions, creams, and suspensions.

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Antibacterial Activity of *Artemisia herba-alba*

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Received July 31, 1978, from the **Department of Microbiological Chemistry, The Hebrew University—Hadassah Medical School, Jerusalem, Israel,* and the ‡*Department of Pharmacognosy, School of Pharmacy, The Hebrew University, Jerusalem, Israel.* Accepted for publication January 12, 1979.

Abstract □ The antibacterial activity of *Artemisia herba-alba* was investigated. Only its essential oil was active against some Gram-positive and Gram-negative bacteria. The essential oil was fractionated by column chromatography, and these fractions were tested for antibacterial activity. The principal component of the most active fraction was santolina alcohol.

Keyphrases □ *Artemisia herba-alba*—antibacterial activity, essential oil, column chromatography □ Antibacterial activity—evaluated for *Artemisia herba-alba* essential oil, column chromatography □ Folk medicine—*Artemisia herba-alba*, antibacterial activity of essential oil

Artemisia herba-alba Asso.[1], known as the desert wormwood, is a dwarf shrub growing in North Africa and the Middle East. This plant is used by the local population as an anthelmintic and for other purposes in folk medicine such as relief of coughing, intestinal disturbances, colds, measles, and muscle weakness.

Recently, several compounds were isolated from the aerial parts of this plant and their structures were established. These compounds were the sesquiterpene lactones

[1] Plant material was collected and identified as *Artemisia herba-alba* Asso. (Compositae) by Dr. Avinoam Danin, Department of Botany, The Hebrew University, Jerusalem, Israel. A voucher specimen (AHA-1) representing material collected for this investigation is available for inspection at the Department of Pharmacognosy (Dr. R. Segal), School of Pharmacy, The Hebrew University, Jerusalem, Israel.

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