thioglycolate to a final concentration of 500 mg/liter (7) to all culture media possibly containing thimerosal. To substantiate the microbial counts and pH or mercury concentrations of effluent samples taken at the end of each wash and rinse cycle, column slurry samples were also analyzed at the conclusion of each antimicrobial effectiveness test.

To monitor the acid wash removal, pH determination was the most appropriate method. Since thimerosal contains ~50% mercury by weight (9), the tromethamine–thimerosal wash removal was monitored by cold vapor, flameless atomic absorption spectroscopy (10).

Washing Solution Effects on Treated Column Separation Properties—The proteins cytochrome c and bovine serum albumin were selected because, based on their respective molecular weights of 12,400 and 67,000, they can be readily fractionated by the slurry used in this investigation (2). Each protein, as well as the blue dextran void volume indicator, was readily assayed spectrophotometrically. In addition, the Ouchterlony double-diffusion procedure, based on a literature method (11), confirmed the clear separation of bovine serum albumin and cytochrome c in the control column and in treated columns.

It is feasible to apply both wash solutions, 0.02 *N* HCl containing 0.81% NaCl and the 0.1 *M* tromethamine–hydrochloride buffer (pH 7.0) containing 0.81% NaCl and 0.02% thimerosal, for disinfecting a modified gel filtration slurry under typical working conditions. Both wash solutions demonstrate antimicrobial activity against high concentrations of various microorganisms in the gel slurry packed into a column maintained at 5°.

Since the acid wash is more effective against nonspore-forming bacteria, including the more resistant species *P. aeruginosa* (4), and since the tromethamine–thimerosal wash is more active against mold and yeast, both wash solutions can be used routinely on an alternating basis or as needed. The wash solutions cannot be used in combination, however, since thimerosal is unstable in acidic solutions (4). In addition, both wash solutions can be removed from the slurry within 24 hr with no more than 1 liter of the rinsing buffer. Both wash solutions do not adversely affect the matrix separation properties and should be compatible with similar column packing materials.

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High-Performance Liquid Chromatographic Analysis of Trimethoprim and Sulfamethoxazole in Dosage Forms

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Abstract □ A rapid, sensitive, and automatable high-performance liquid chromatographic method is presented for the determination of sulfamethoxazole, trimethoprim, and a preservative in dosage forms in the presence of excipients and degradation products.

Keyphrases □ Trimethoprim—simultaneous analysis with sulfamethoxazole and methylparaben, high-performance liquid chromatography, oral suspension and solid dosage forms □ Sulfamethoxazole—simultaneous analysis with trimethoprim and methylparaben, high-performance liquid chromatography, oral suspension and solid dosage forms □ Antibacterial agents—trimethoprim and sulfamethoxazole, simultaneous high-performance liquid chromatographic analysis, oral suspension and solid dosage forms

The antibacterials sulfamethoxazole and trimethoprim are the active ingredients in several oral suspension and solid dosage forms. The official analyses of their dosage forms are spectrophotometric methods following extraction (1, 2). These methods are time consuming and relatively difficult. A high-performance liquid chromatographic (HPLC) method, presented for the analysis of various sulfonamides in combination with trimethoprim, does not separate the various degradation products and the active components and cannot be used as a stability-indicating assay (3).

This study was undertaken to establish a rapid, quantitative, and stability-indicating procedure for routine quality control testing of trimethoprim and sulfamethoxazole in dosage forms. Methylparaben, a commonly used preservative, also may be determined in the analysis of the oral suspension.

EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[1] with a 254-nm detector and a stainless steel column (30 cm × 4 mm i.d.) was used. The column packing[2] was porous silica particles with an octadecylsilane-bonded coating. The system was operated at 2.0 ml/min with a column pressure of ~1800 psi for solid dosage forms and at 3.0 ml/min with a column pressure of ~2800 psi for oral suspensions.

[1] Model 6000A pump and 440 detector, Waters Associates, Milford, Mass.
[2] μBondapak C_{18}, Waters Associates, Milford, Mass.

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Samples were injected by an autosampler[3] equipped with a 10-μl loop, and all analyses were performed at ambient temperature. A laboratory computer system[4] was used to gather data and to calculate the percentage of the theoretical component amounts.

Materials—Methanol[5], methylparaben[6], phenacetin[6], trimethoprim[6], sulfamethoxazole[6], acetonitrile[7], and acetic acid[8] were used as received. The oral suspensions and tablets were obtained from marketed batches[9].

Mobile Phase—The mobile phase was degassed acetonitrile-1% aqueous acetic acid (16:84) for tablets and degassed acetonitrile-1% aqueous acetic acid (10:90) for oral suspensions.

Internal Standard Solution—A 2-mg/ml solution of phenacetin in methanol was prepared as the internal standard.

Reference Standard Solution—Approximately 20 mg of trimethoprim and 100 mg of sulfamethoxazole were weighed accurately into a 100-ml volumetric flask. After 6.0 ml of the internal standard was added and the trimethoprim and sulfamethoxazole were dissolved in methanol[10], the flask was filled to volume with methanol.

Sample Preparation for Solid Dosage Forms—Twenty intact tablets from each sample were weighed accurately to obtain the average tablet weight and were ground to a fine powder. An amount of the powder equivalent to 100 mg of sulfamethoxazole was weighed accurately and placed in a 100-ml volumetric flask. Sufficient methanol to dissolve the active ingredients[10] and 6.0 ml of the internal standard solution were added, and the solution was diluted to volume with methanol. A portion of this solution was centrifuged or filtered before use.

Sample Preparations for Oral Suspension—Approximately 2.7 g of the oral suspension sample was weighed accurately and placed in a 100-ml volumetric flask. After 6.0 ml of the internal standard solution was added and the active ingredients were dissolved in methanol[10], the flask was diluted to volume with methanol. A portion of this solution was filtered or centrifuged before use. The specific gravity of the suspension was required for the calculations.

Chromatography—Each sample analysis consisted of two 10-μl injections of the sample solution bracketed by two 10-μl injections of a standard solution. Sample chromatograms for the oral suspension and tablet samples are shown in Figs. 1 and 2, respectively. The detector attenuation was 0.1 aufs.

The peak area or height ratios were used to quantitate the chromatograms. The ratios were calculated by:

$$SP \text{ or } ST = \frac{PA_1}{PA_2} \qquad \text{(Eq. 1)}$$

where SP is the sample ratio, ST is the standard ratio, PA_1 is the area or height of the sample or standard component peak, and PA_2 is the area or height of the internal standard peak. The percent of the theoretical amount of each component was calculated by:

$$\left(\frac{SP}{ST}\right)\left(\frac{W_1}{W_2}\right)\left(\frac{AS}{TA}\right) \times 100 = \% \text{ of theoretical amount} \qquad \text{(Eq. 2)}$$

where W_1 and W_2 are the standard and sample weights, respectively; AS is the average tablet weight or specific gravity; and TA is the theoretical amount per tablet or milliliter. Peak areas were used in the trimethoprim and sulfamethoxazole calculations, and peak heights were used in the methylparaben calculations.

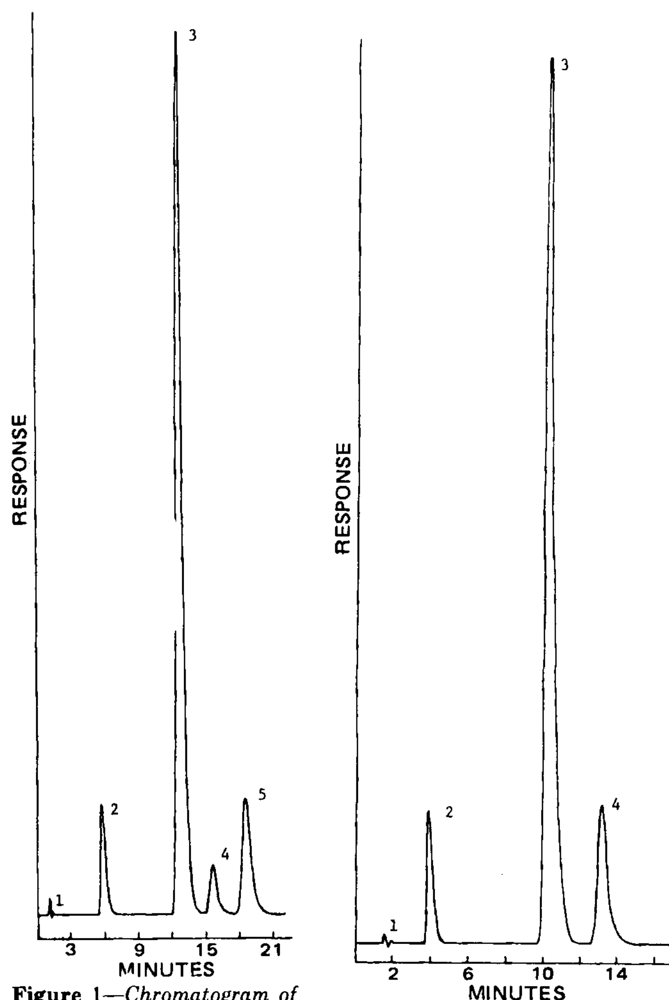

Figure 1—*Chromatogram of an oral suspension. Key: 1, solvent; 2, trimethoprim; 3, sulfamethoxazole; 4, methylparaben; and 5, phenacetin.*

Figure 2—*Chromatogram of a solid dosage form. Key: 1, solvent; 2, trimethoprim; 3, sulfamethoxazole; and 4, phenacetin.*

RESULTS AND DISCUSSION

A nonpolar column was selected due to the polarity of the components to be determined. The high efficiency of the reversed-phase, small particle column resolved all of the formulation components and potential degradation products.

The presence of methylparaben as a preservative in the oral suspension required a different mobile phase from that used for the tablets. A slightly more polar mobile phase was used to resolve methylparaben from sulfamethoxazole in the oral suspension to quantitate both components accurately. The less polar mobile phase allowed faster analysis for the tablets.

An internal standard method was chosen to minimize errors resulting from both the apparatus and the injection technique. The peak exhibited by phenacetin, the internal standard, was resolved well from the peaks exhibited by trimethoprim, sulfamethoxazole, methylparaben, and the degradation products.

Linearity of the method for sulfamethoxazole and trimethoprim was determined by plotting the peak area response *versus* the concentration of each component. Peak height response was plotted *versus* concentration for the methylparaben linearity determination. Solutions containing various amounts of the component and a constant amount of the internal standard were chromatographed. The results proved that the method was linear in accordance with Beer's law.

Method reproducibility was determined by 13 consecutive injections of a standard solution equivalent to 100% of the theoretical amount of each component. The relative standard deviations were 0.16, 0.36, and 5.30%, using the peak area for sulfamethoxazole, trimethoprim, and methylparaben, respectively. The relative standard deviation for methylparaben was 0.97% using peak height measurements. Since this reproducibility is greater than that obtained using peak area, peak heights were used for the methylparaben calculations.

Each component was heat degraded in acid, base, and water. These solutions were chromatographed to show the retention times for the degradation products. Sulfamethoxazole exhibited no noticeable degradation, and trimethoprim exhibited two peaks identified as 2-amino-4-hydroxy-5-(3′,4′,5′-trimethoxybenzyl)pyrimidine and 4-amino-2-hydroxy-5-(3′,4′,5′-trimethoxybenzyl)pyrimidine. The retention times of the two degradation products using the mobile phase for solid dosage

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[3] Model 725, Micromeritics, Norcross, Ga.
[4] HP3354, Hewlett-Packard, Avondale, Pa.
[5] Certified ACS, Fisher Scientific.
[6] Reference standards, Burroughs Wellcome Co., Greenville, N.C.
[7] HPLC grade, Fisher Scientific.
[8] ACS grade, Mallinckrodt.
[9] Septra suspension and Septra tablets, Burroughs Wellcome Co., Greenville, N.C.
[10] Samples or standards may have to be sonicated or shaken to effect dissolution.

Table I—Comparative Analyses Using HPLC and Extraction–Spectrophotometric Methods

Sample	Percent of Theoretical Amount[a]					
	Trimethoprim		Sulfamethoxazole		Methylparaben	
	Extraction	HPLC	Extraction	HPLC	Previous[b]	HPLC
1	96.6	99.8 ± 0.7	100.6	100.1 ± 0.0	—	—
2	98.7	100.0 ± 0.2	98.0	100.6 ± 0.1	—	—
3	99.8	99.7 ± 0.3	98.9	99.5 ± 0.7	—	—
4	99.4	98.8 ± 0.4	100.6	99.4 ± 0.0	—	—
5	97.5	95.1 ± 0.2	102.7	98.0 ± 0.2	—	—
6	101.8	100.4 ± 0.6	100.3	100.0 ± 0.8	—	—
7	101.0	99.5 ± 0.2	100.3	99.5 ± 0.3	—	—
8	102.1	97.7 ± 0.1	101.7	100.1 ± 0.3	—	—
Suspension 1	96.6	100.1 ± 1.5	99.8	102.2 ± 0.8	100.5	99.8 ± 0.4
Suspension 2	97.6	97.5 ± 0.3	101.5	100.0 ± 0.4	97.4	100.1 ± 0.0
Suspension 3	94.1	95.6 ± 0.8	101.5	99.4 ± 0.6	99.8	99.3 ± 0.8
Suspension 4	97.3	97.0 ± 1.1	99.3	99.9 ± 0.8	97.8	99.6 ± 0.0
Suspension 5	98.6	96.4 ± 1.1	97.6	100.4 ± 0.4	98.6	99.5 ± 0.6
Suspension 6	95.2	95.4 ± 1.3	98.9	98.7 ± 0.7	97.0	97.2 ± 0.6

[a] Each HPLC result is an average of two values. The percent of the theoretical amount is the amount of active ingredient found based on the amount claimed. [b] The previous assay was a different HPLC assay that used an acetonitrile–0.1% acetic acid (25:75) mobile phase and an ODS-2, 25-cm column at a flow rate of 2.0 ml/min.

Table II—Reproducibility of HPLC Method Results

Sample	Percent of Theoretical Amount[a]					
	Trimethoprim		Sulfamethoxazole		Methylparaben	
	Assay I	Assay II	Assay I	Assay II	Assay I	Assay II
1	99.8 ± 0.7	99.4 ± 0.4	100.1 ± 0.0	100.5 ± 1.1	—	—
2	95.1 ± 0.2	96.4 ± 0.3	98.0 ± 0.2	99.8 ± 0.4	—	—
3	100.4 ± 0.6	100.6 ± 0.6	100.0 ± 0.8	101.6 ± 0.4	—	—
Suspension 1	95.0 ± 0.1	95.3 ± 0.5	98.0 ± 0.1	98.7 ± 0.2	98.0 ± 0.3	99.2 ± 0.1
Suspension 2	94.2 ± 0.1	94.1 ± 0.4	99.3 ± 0.3	99.1 ± 0.6	99.3 ± 0.6	98.5 ± 0.5
Suspension 3	94.5 ± 0.2	94.5 ± 0.1	98.7 ± 0.0	98.3 ± 0.1	98.7 ± 0.2	97.7 ± 0.4

[a] Each assay result is an average of two analyses. Samples were weighed for each analysis. The percent of the theoretical amount is the amount of active ingredient found based on the amount claimed.

forms were ~4.9 and 3.1 min, respectively. The retention times using the mobile phase for the oral suspension were ~10.0 and 4.2 min, respectively. These degradation products were identified by chromatographing standard solutions containing the impurities, the degradation solutions containing the products of interest, and degradation solutions spiked with these products and noting the retention times of the peaks. Placebos were chromatographed and showed that the excipients did not interfere.

The values obtained using the HPLC method compared favorably with those obtained using the extraction–spectrophotometric determination (Table I). In most cases, the HPLC assay gave slightly higher values than the extraction method. This difference may have been caused by a loss of sample during the extraction. In some cases, absorbing impurities or degradation products may have caused the extraction–spectrophotometric method to give a higher result than the more specific HPLC method. The HPLC assay was performed on 2 days and showed that the precision of the instrumentation and the assay reproducibility results were good. A different sample was weighed each day the assay was performed (Table II).

The HPLC method is accurate and is shorter and easier than the extraction–spectrophotometric method. In addition, the HPLC method can be automated easily using automatic samplers and laboratory computers or integrators.

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