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QUANTITATION OF SULFACETAMIDE, SULFADIAZINE, SULFAMERAZINE AND SULFAMETHAZINE IN VARIOUS COMBINATIONS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A reverse phase high-performance liquid chromatography method for the quantitation of sulfacetamide, sulfadiazine, sulfamerazine, and sulfamethazine in various combinations has been developed. The method is simple, accurate, precise and reproducible. The percent relative standard deviations based on 6 injections were 2.1, 0.6, 1.9, and 1.6 for sulfacetamide, sulfadiazine, sulfamerazine, and sulfamethazine, respectively. The ratio of peak heights (drug/internal standard) were closely related (r value 0.99 or better) to concentrations (± 20% of the standard solution concentrations). The results of synthetic mixtures showed quantitative recovery and method was successfully applied to commercial dosage forms (tablets and suspension). Extraction of sulfa drugs from the dosage forms required a very simple procedure.

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BACKGROUND

Many sulfa drugs are used extensively in medicine. Besides single ingredient tablets/suspension/creams, the sulfas are available in various combinations. The most commonly available combination for oral use contains equal quantities of sulfadiazine, sulfamerazine, and sulfamethazine. This combination is available commercially as tablets or suspension. The other less common combination for oral use is of sulfacetamide, sulfadiazine, and sulfamerazine.

The most commonly used methods for the quantitation of sulfas were reviewed by Fatmi et al[1]. These authors also proposed a HPLC method for the quantitation of sulfacetamide, sulfabenzamide and sulfathiazole in combination in a cream. The USP-NF[2] methods for the quantitation of sulfa drugs are based on either sodium nitrite titration or TLC/UV spectroscopy which are not specific. The combination of sulfadiazine, sulfamerazine and sulfamethazine is not official. The quantitation of sulfa drugs in this combination using a cation-exchange column has been reported[3].

The purpose of these investigations was to develop a reverse phase high-performance liquid chromatography method for the quantitation of sulfacetamide (I), sulfadiazine (II), sulfamerazine (III), and sulfamethazine (IV) in various combinations.

MATERIALS AND METHODS

Chemicals and Reagents: All the chemicals and reagents were either USP/NF or ACS grade and used without further purification. The tablets and suspension were from commercial lots[4].

Apparatus: A high-performance liquid chromatograph[5] equipped with a multiple wavelength detector[6] and a recorder[7] was used.

Column: A non-polar column[8] (30 cm x 3.9 mm i.d.) was used.

Chromatographic Conditions: The mobile phase contained 16% (v/v) of methanol and 0.02 M KH_2PO_4 in water. The flow rate was 2.0 ml/min and the temperature was ambient. The sensitivity was 0.1 (0.04 for sulfamethazine when quantifying in combination with sulfadiazine and sulfamerazine) at 257 nm. The chart speed was 30.5 cm/hr.

Preparation of Stock Solutions: A 80.0 mg quantity of each sulfa powder was dissolved in 2 ml of a ~1 N NaOH solution and brought to volume (100.0 ml) with water. A 4.0 ml portion of each stock solution was mixed with equal volume of ~0.1 M KH_2PO_4 solution and brought to volume (100.0 ml) with water. The standard mixtures of sulfa drugs were prepared similarly as needed. Before bringing to volume, the stock solution of the internal was added. The quantities of sulfacetamide and sulfamethazine added were 2.0 and 8.0 ml, respectively versus 4.0 ml of each sulfa drug to be assayed. The mixtures of sulfa drugs containing other concentrations were prepared as needed by diluting different volumes of the stock solutions.

Preparation of Assay Solutions: For tablets (166.7 mg per tablet of each sulfa): Ten tablets were ground to a fine powder. A portion of the powder representing 40.0 mg of all the 3 sulfas combined were weighed accurately and dissolved in 1 ml of ~1 N NaOH solution. The mixture was brought to volume (50.0 ml) with water

and filtered[9]. First 10 ml of filtrate was rejected and then some collected for further dilution. A 6.0 ml portion of the filtrate was mixed with 1.0 ml portion of the stock solution of sulfacetamide (internal standard) and 7 ml of ∿0.1 M KH_2PO_4 solution and brought to volume (50.0 ml) with water. All the synthetic mixtures were also treated same way except that to assay mixtures containing sulfacetamide, sulfamethazine was used as the internal standard (4.0 ml of the stock solution) and the quantity of ∿0.1 M KH_2PO_4 solution was increased to 10 ml.

For suspensions (166.7 mg/5 ml of each sulfa): A 10 ml portion of the suspension was mixed with 10 ml of ∿1 N NaOH solution and brought to volume (500.0 ml) with water. A 20.0 ml portion of this mixture was brought to 50.0 ml with water. A 6.0 ml portion of this solution was mixed with 1.0 ml of the stock solution of sulfacetamide, 7 ml of ∿0.1 M KH_2PO_4 solution and brought to volume (50.0 ml) with water.

Assay Procedure: A 20 µl of the assay solution was injected into the chromatograph using the described conditions. For comparison, an identical volume of the standard dilution was injected after the assay solution eluted.

Calculations: Since preliminary investigations indicated that the ratio of peak heights (sulfa/internal standard) were directly related to the concentrations (range tested, ± 20% of the concentrations in the standard solution), the results were calculated using the equation:

$$\frac{(Ph)_a}{(PH)_s} \times 100 = \text{Percent of the label claim}$$

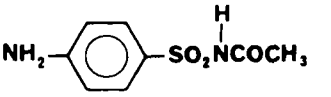
TABLE 1

Assay Results

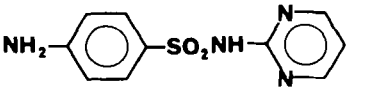
Dosage Form or Synthetic Mixture	Label Claim	Percent of the Label Claim Found: Sulfacetamide (I)	Sulfadiazine (II)	Sulfamerazine (III)	Sulfamethazine (IV)
Tablets	166.7 mg each of II, III and IV	-[b]	102.9	100.1	100.0
Suspension	166.7 mg[a] each of II, III and IV per 5 ml.	-[b]	105.1	104.4	99.9
Synthetic Mixture #1	166.7 mg each of II, III and IV plus 150 mg of dextrose	-[b]	99.9	99.1	98.8
Synthetic Mixture #2	166.7 mg each of II, III and IV plus 150 mg of lactose	-[b]	101.2	100.6	99.6
Synthetic Mixture #3	166.7 mg each of I, II and III plus 150 mg of dextrose	100.5	99.7	100.8	-[b]
Synthetic Mixture #4	166.7 mg each of I, II and III plus 150 mg of lactose	100.6	99.5	99.6	-[b]

[a]Also contained 0.05% each of methylparaben and propylparaben.
[b]Not present in this combination.

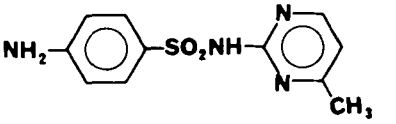
Sulfacetamide



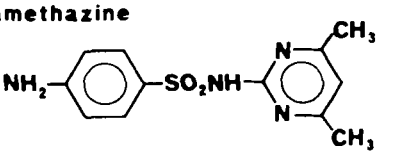
Sulfadiazine



Sulfamerazine



Sulfamethazine



Sulfapyridine

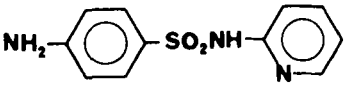


FIGURE 1

Structures of sulfas studied.

where $(Ph)_a$ is the ratio of the peak heights of assay solution and $(Ph)_s$ that of the standard solution.

RESULTS AND DISCUSSION

The results indicated (Table 1 and Figure 2) that the developed HPLC method can be used to quantify sulfacetamide, sulfadiazine, sulfamerazine, and sulfamethazine either as a single ingredient or in various combinations. The method is accurate (Table 1) and pre-

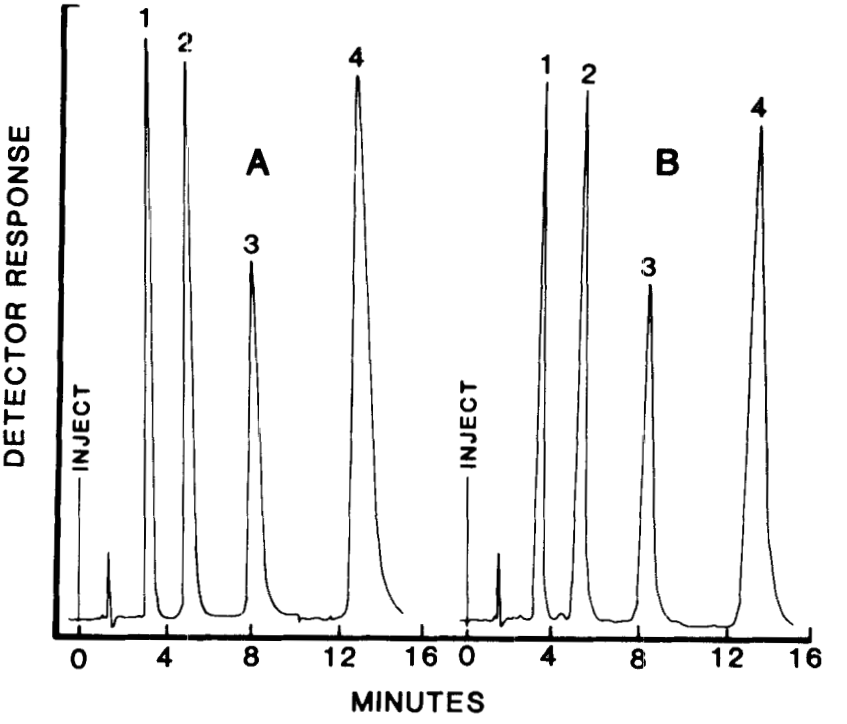


FIGURE 2

Sample chromatograms. Peaks 1-4 are from sulfacetamide, sulfadiazine, sulfamerazine, and sulfamethazine, respectively. Chromatogram A is from a standard solution and B from the suspension. For chromatographic conditions, see text.

cise with a percent relative standard deviations based on 6 readings of 2.1, 0.6, 1.9, 1.6, for sulfacetamide, sulfadiazine, sulfamerazine and sulfamethazine, respectively. In the most commonly used combination (equal quantities of sulfadiazine, sulfamerazine and sulfamethazine), the separation of the internal standard (sulfacetamide) was complete (Figure 2). When sulfapyridine was used as the internal standard, the separation from sulfamerazine (Figure 3) was not complete. Therefore, sulfacetamide was prefer-

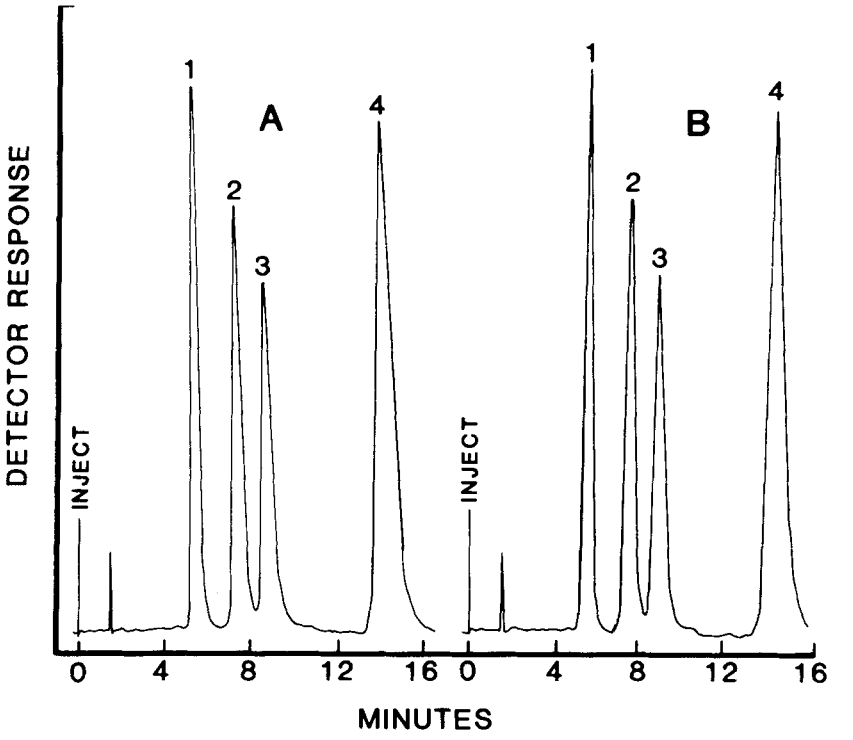


FIGURE 3

Sample chromatograms. Peaks 1-4 are from sulfadiazine, sulfapyridine, sulfamerazine, and sulfamethazine, respectively. Chromatogram A is from a standard solution and B from tablets. For chromatographic conditions, see text.

red as the internal standard for combination of II, III, and IV sulfa drugs. For the combination of I, II and III sulfa drugs, sulfamethazine was used as the internal standard. The result of synthetic mixtures were quantitative and showed complete recovery (Table 1). The ratio of the peak heights (drug/ internal standard) were directly related to the concentrations. Within a range of 20% of the standard solution, the correlation coefficient (r value) was 0.99 or better.

The method developed was successfully tried to quantify II, III, and IV sulfa drugs in commercial dosage forms (tablets and suspension). The results were excellent (Table 1). In the case of suspension, the results were slightly different each time due to difficulty in taking a good uniform sample. There was no interference from the preservatives (0.05% each of methylparaben and propylparaben) as determined by injecting the pure samples of these compounds. The other excipients were not disclosed on the labels. The developed procedure for the preparation of the assay samples from tablets and supension is very simple.

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4. Terfonyl® lots 5B269 (tablets) and 4A123 (suspension) by E.R. Squibb & Co., Princeton, NJ.
5. Waters ALC 202 equipped with U6K universal injector, Waters Associates, Milford, MA.
6. Schoeffel Spectroflow Monitor 770, Kratos Inc., Ramsey, NJ.
7. Omniscribe 5213-12, Houston Instruments, Austin, TX.
8. Waters Microbondapak/C_{18}, Waters Associates, Milford, MA.
9. Fisher's 9-801E, Fischer Scientific, Fairlawn, NJ.