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# DETERMINATION OF SULFONAMIDES IN TRIPLESULFA CREAM BY HPLC METHOD

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## ABSTRACT

A fast, accurate, economical, and reproducible HPLC procedure has been developed to quantitate sulfacetamide (I), sulfabenzamide (II), and sulfathiazole (III) in vaginal creams, using sulfapyridine (IV) as internal standard. The method employed u Bondapak phenyl column, Waters Liquid Chromatograph, Hewlett-Packard 3390A reporting integrator and UV detector set at 280 nm. The mobile phase consisted of 7:3 mixture of 0.01 M ammonium hydrogen phosphate and methanol (pH 7.2). The method met USP requirements for system suitability and linearity (R=0.998). The USP method consistently gave low and widely varying results ranging from 75 to 102% for sulfacetamide. The results for sulfabenzamide and sulfathiazole were comparable by both methods.
The relative retention time for I, II, III, IV were 0.4, 0.5, 0.9, and 1.0 respectively for the proposed

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method and 0.8, 2.5, 1.8, and 1.0 for the USP method. The USP method was tedious and unreliable for assay of (I). Considering the cost of the column and mobile phase, the proposed method was 70% less costly than the USP mehtod.

## INTRODUCTION AND BACKGROUND

Creams containing triplesulfas namely Sulfacetamide (I), Sulfabenzamide (II) and Sulfathiazole (III) have recently been introduced for the treatment of Haemophilus Vaginalis. This paper reports the analysis of Sulfonamides in three commercial products using High Performance Liquid Chromatography.

Mixtures containing more than one Sulfa drug were traditionally analysed by paper and thin layer chromatography (1-4). Often these chromatographic separations are followed by Bratton and Marshall procedure (5-8).

Use of Gas Chromatography (GC) in the analysis of Sulfonamides has been reported (9-12). High Performance Liquid Chromatography (HPLC) assay methods have been reported for various Sulfonamides (13). The current USP assay for Triple Sulfa creams is also performed by using HPLC method (14).

In our hands the USP method gave inconsistent results for Sulfacetamide. The proposed HPLC procedure not only gave consistent results for Sulfacetamide, but was quite compatible with the USP method in every respect.

## EXPERIMENTAL

### Reagents And Apparatus

The methanol,[1] acetone,[2] ammonium hydrogen phosphate,[3] phosphoric acid[4] and, ammonium hydroxide[5] were used as received. All chemicals were analytical reagent grade.

All HPLC analyses were carried out using a liquid chromatograph[6] equipped with an autosampler,[7] and a variable wavelength detector[8] set at 280-nm wavelength. Peak areas were measured by an integrator.[9] A 25-cm x 4.6-mm i.d. Column[10] was used.

Preparation Of Mobile Phase

The mobile phase consisted of a 3:7 mixture of methanol, .01M ammonium hydrogen phosphate buffer solution. The pH was adjusted to 7.2 ± 0.1 with either phosphoric acid or ammonium hydroxide. The mobile phase was filtered through a memberane filter[11] (pore size 0.45 um) and deaerated prior to use.

Preparation Of Internal Standard

USP Sulfapyridine Reference Standard was dissolved in acetone to obtain a solution having a concentration of 10 mg/ml.

Preparation Of Standard Solutions

Accurately weighed quantities of USP Sulfacetamide Reference Standard (39 mg), USP Sulfabenzamide Reference Standard (37 mg) and USP Sulfathiazole Reference Standard (34 mg) were transferred to a 50 ml volumetric flask. A 2.0 ml of Internal standard solution and 30 ml of acetone were added, shaken for 10 minutes and sonicated, if necessary, to affect solution. The solution was then made to volume with acetone.

A 5 ml portion of this solution was pipetted into a 100 ml volumetric flask, evaporated to dryness on a steam bath with a gentle stream of nitrogen. The residue was reconstituted in mobile phase.

Preparation Of Sample Solution

Using a plastic syringe equipped with a suitable cannula, an accurately weighed quantity of Sulfacetamide, (I) Sulfabenzamide (II) and Sulfathiazole (III) cream, equivalent to about 144 mg of (I), 184 mg of (II) and 173 mg of (III) were transferred to a 250 ml volumetric flask. A 10 ml portion of internal standard and 100 ml of acetone were added. The flask was warmed on a steam bath while swirling the contents to dissolve the cream.

After cooling the solution to room temperature and diluting it to volume with acetone, the solution was filtered through a membrane filter[11] (pore size 0.45 um) discarding the first 10 ml of the filtrate. A 5 ml portion of this filtrate was pipetted into a 100 ml volumetric flask, evaporated to dryness on a steam bath with the aid of a gentle stream of nitrogen.
The residue was reconstituted in mobile phase. The solution so obtained was cooled in an ice bath for 10 minutes, filtered through membrane filter[11] (pore size 0.45 mu) discarding the first 10 ml of the filtrate. About 6 ml of this solution was collected for assay.

All solutions were prepared in accordance with USP procedure (14).

## Procedure Of Assay

The HPLC system was equilibrated by passing mobile phase through the column for 0.5 hr. A 20 ul portions of standard preparation were injected five times followed by triplicate 20 ul injections of Sample preparation. Typical relative retention times were:Sulfacetamide 0.4, Sulfabenzamide 0.5, Sulfathiazole 0.9 and Sulfapyridine 1.0 minutes.

The quantities of Sulfonamides were calculated using the equation:

$$\text{mg of Sulfonamides} = \frac{Ru}{Rs} \times C \times D.F.$$

where: $\frac{Ru}{Rs}$ = ratios of the peak responses of the corresponding Sulfonamides to those of the internal standard obtained from the sample solution and standard solution respectively.

C = Concentration, ug/ml of appropriate reference standards.

DF = dilution factor.

System Suitability

The HPLC system was equilibrated by passing mobile phase through the column for 0.5 hr, portions of the standard solutions (20 ul) were then injected. The system was considered suitable when:

The retention times for I, II, III and IV were 2.0, 3.0, 4.91, and 5.75 minutes respectively.

The resolution factor was not less than 2.0

The column efficiency N calculated using the III peak was not less than 5000 theoretical plates.

The relative standard deviations of the response ratios of I, II, III peaks relative to the internal standard IV peak for six consecutive injections did not exceed 3.0%

## RESULTS AND DISCUSSION

Initial attempts to adopt USP procedure for the assay of triple Sulfa Vaginal Cream consistenly failed in our hands. The USP procedure employs a $C_{18}$ Column with water, acetonitrile and tetrabutyl ammonium phosphate as mobile phase yielding an assay that gives inconsistent results for Sulfacetamide (Table 1).

Experiments with more polar phenyl bonded column were initiated ang good results were obtained (Table 2). The three Sulfas were well separated with retention times of 2.0, 3.0, and 4.9 for Sulfacetamide (I) Sulfabenzamide (II) and Sulfathiazole (III) respectively (Figure 2).

The linearity of response for I, II and III was determined by plotting peak area ratios versus standard concentrations ratios (mg/ml) to internal

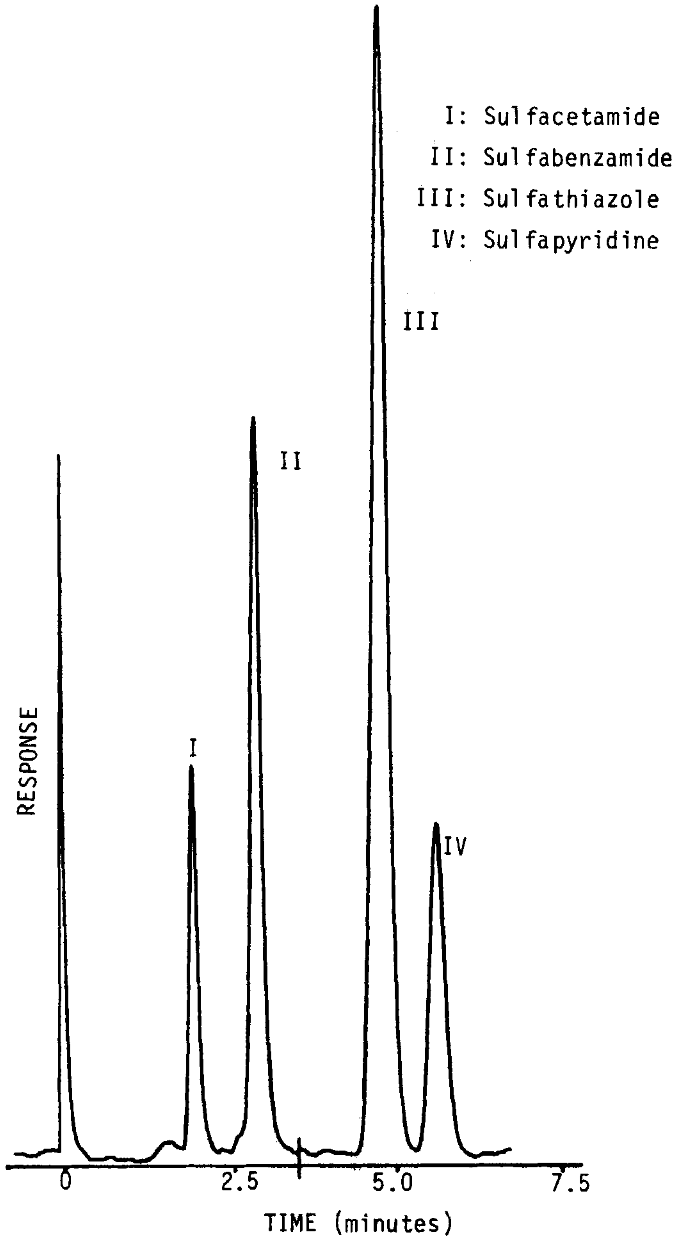

FIGURE 1. A Typical HPLC Chromatogram of standard/ Internal Standard

TABLE 1

ASSAY OF TRIPLESULFAS BY USP METHOD
(HPLC)

| | PERCENT OF LABEL CLAIM [a] | | |
|---|---|---|---|
| | Product A | Product B | Product C |
| Sulfacetamide | 75-85 | 87-96 | 89-102 |
| Sulfabenzamide | 100.2±1.1 | 103.6±1.21 | 101.8±1.09 |
| Sulfathiazole | 106.1±2.7 | 100.4±2.1 | 102.6±3.0 |

a: Label claim for Sulfacetamide: 28.7 mg., Sulfabenazmide: 37.0 mg., and Sulfathiazole: 34.2 mg. in each product.

TABLE 2

ASSAY OF TRIPLESULFAS USING PROPOSED
(HPLC) METHOD

| | PERCENT OF LABEL CLAIM | | |
|---|---|---|---|
| | Average of Assay for Product A | Average of Assay for Product B | Average of Assay for Product C |
| Sulfacetamide | 100.70±1.04 | 102.9±0.98 | 100.4±1.03 |
| Sulfabenzamide | 104.32±1.3 | 108.7±1.18 | 106.3±1.27 |
| Sulfathiazole | 103.5±3.2 | 106.1±2.5 | 102.5±2.36 |

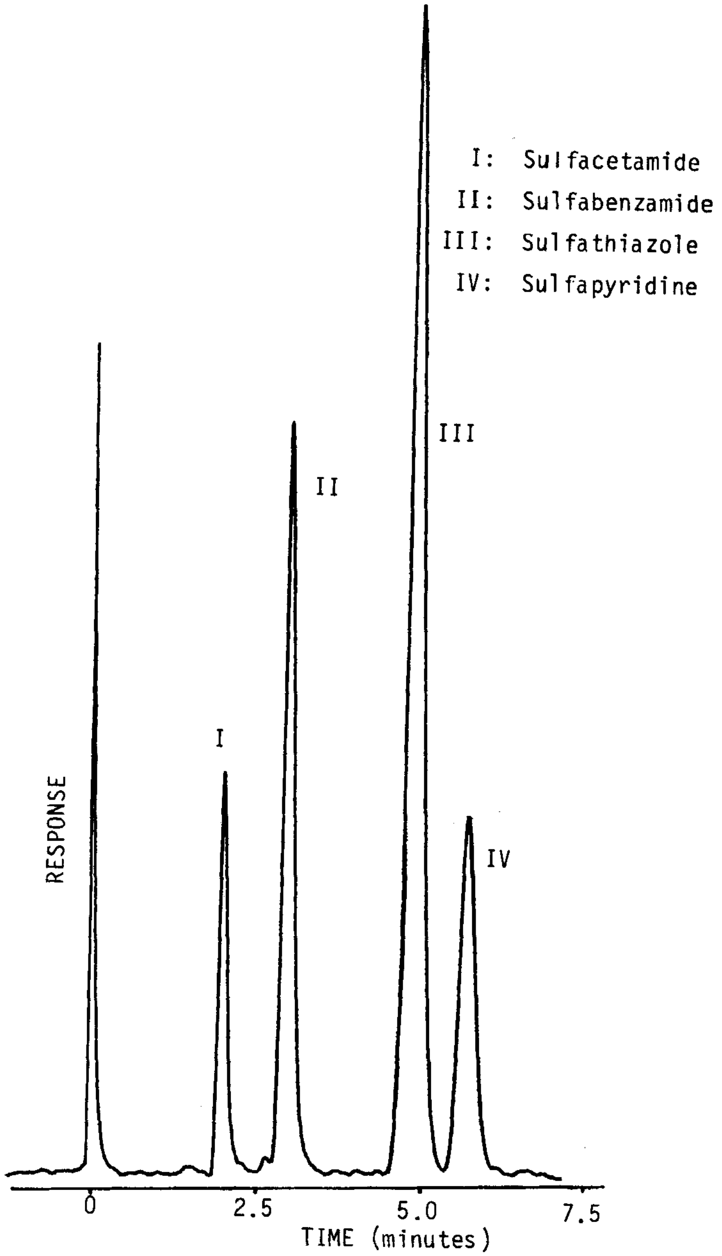

FIGURE 2. A Typical HPLC Chromatogram Of A Triplesulfa Cream.

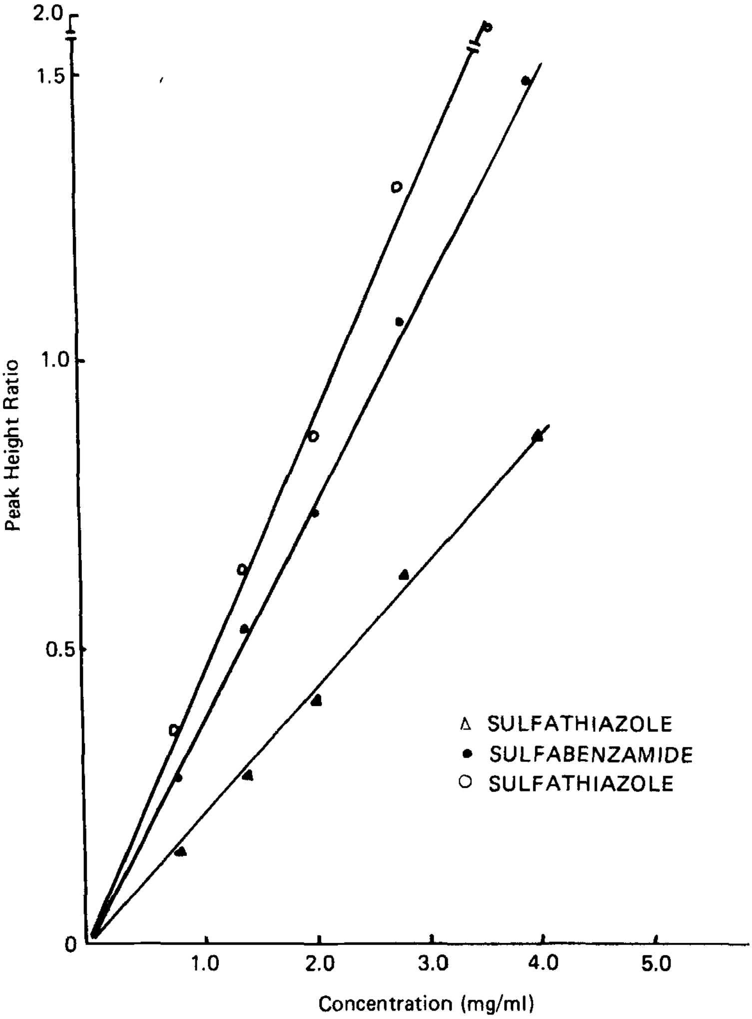

FIGURE 3. Calibration Curve

standard concentration (Figure 3). For Sulfacetamide (I) the detector response was linear to atleast 0.5 mg/ml with a correlation coefficient of 0.998. For Sulfabenzamide (II) and Sulfathiazole (III) the detector response was linear to atleast 0.25 mg/ml with a correlation coefficient of 0.999.

Recovery from standard addition of active ingredients to base cream at levels of 90-110% of label claim

## TABLE 3

## STANDARD ADDITION AND RECOVERY DATA*
## FOR TRIPLE SULFA CREAM

| Name of Drug | Assay Level | Amount Added (mg) | Amount Recovered (mg) | %Recovered |
|---|---|---|---|---|
| Sulfacetamide | 90% | 25.7 | 25.5 | 99.22 |
| | 100% | 28.6 | 28.8 | 100.70 |
| | 120% | 34.3 | 34.7 | 101.2 |
| Mean | | | | 100.37 |
| SD | | | | ±1.03 |
| Sulfabenzamide | 90% | 33.3 | 34.5 | 103.6 |
| | 100% | 37.0 | 38.8 | 104.32 |
| | 120% | 44.4 | 45.2 | 101.8 |
| Mean | | | | 103.23 |
| SD | | | | ±1.29 |
| Sulfathiazole | 90% | 30.8 | 31.6 | 102.6 |
| | 100% | 34.2 | 35.4 | 103.5 |
| | 120% | 41.0 | 41.0 | 97 |
| Mean | | | | 101 |
| SD | | | | ±3.52 |

* (i) All samples were prepared in triplicate for each assay level in Triplesulfa Cream Base.

(ii) The amount recovered of each drug is an average of triplicate runs.

TABLE 4

CONTENT UNIFORMITY OF THREE SULFONAMIDES

| Number of Tubes Assayed | Sulfacetamide % Label | Sulfabenzamide % Label | Sulfathiazole % Label |
|---|---|---|---|
| 1 | 101 | 102 | 106 |
| 2 | 99.4 | 110 | 106 |
| 3 | 99.4 | 105 | 96.5 |
| 4 | 98.6 | 107 | 102.0 |
| 5 | 95.7 | 104.8 | 100.9 |
| 6 | 102.6 | 103.5 | 108.6 |
| 7 | 100.8 | 100.9 | 103.4 |
| 8 | 102.2 | 104.6 | 99.9 |
| 9 | 104.3 | 100.2 | 107.6 |
| 10 | 102.1 | 108.5 | 107.3 |
| Average ±S.D. | 100.6±2.44 | 104.6±3.18 | 103.8±3.94 |

yielded average percent recoveries of 100.37 ± 1.03 for (I), 1.03.23 ± 1.29 for (II) and 101 ± 3.52 for (III) (Table 3).

Content uniformity assays of 10 samples gave average recoveries of 100.6 ± 2.44 (I), 104.6 ± 3.18 (II) and 103.8 ± 3.94 (III). (Table 4).

The assay results for three commercial products (A, B and C) are presented in Table 1 using USP-HPLC procedure and Table 2 using proposed HPLC procedure. As results in Table 2 indicate, the proposed procedure is highly reproducible, efficient, economical and comparable to USP method.

## ACKNOWLEDGEMENT

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## FOOTNOTES

1. Omnisolv, MCB.
2. J.T. Baker Chemical Company.
3. Fisher Scientific.
4. Fisher Scientific.
5. Fisher Scientific.
6. Model 6000, Waters Associates.
7. Model 710B, Waters Associates.
8. Model 480, Waters Associates.
9. Model 3390A, Hewlett Packard.
10. u-bondapak phenyl, Waters Associates.
11. Millipore Corporation.

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