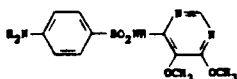


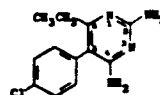
## QUANTITATION OF ANTI-MALARIAL DRUGS USING $\mu$ BONDAPAK™ C<sub>18</sub> COLUMN

Fansidar® (Hoffman-La Roche, Switzerland) is frequently used for the prophylaxis and treatment of malaria. Each tablet of Fansidar® contains 500 ng sulphadoxine [4-Amino-N-(5,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide] (SULPH; I) and 25 ng pyrimethamine [5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine] (PYR; II). SULPH and PYR act synergistically to block enzymes in plasmodial pyrimidine synthesis. Analytical methodologies proposed for the analysis of this preparation have suffered from the standpoint of the inability to quantitate these two compounds simultaneously. Recently, however, an article has appeared in the literature describing a simple, selective, and sensitive HPLC method for simultaneously separating and quantitating both SULPH and PYR, and also a major metabolite of SULPH, N<sub>4</sub>-Acetylsulphadoxime (NASULPH) in human plasma using a Waters  $\mu$ BONDAPAK™ C<sub>18</sub> column to effect the separation (1).

I. Sulphadoxine (SULPH)  
4-Amino-N-(5,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide



II. Pyrimethamine (PYR)  
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine



The sample was prepared by extracting an internal standard-spiked plasma sample with ethylene dichloride. This organic phase was isolated, dried, and reconstituted in mobile phase, from which 40  $\mu$ l injections were made. Extraction recoveries averaged 80% for these compounds. The mobile phase, consisting of methanol:acetonitrile:water (25:15:60) containing 0.005M 1-pentane sulphonic acid (PIC® B-5, pH 3.40) was pumped at a flow rate of 1.5 ml/min. The column used was a 30 cm by 3.9 mm I.D.  $\mu$ BONDAPAK™ C<sub>18</sub> with a particle size of 10  $\mu$ M.

The separation obtained is illustrated in Figure 1. No significant interfering peaks were present in drug free plasma extract at the retention time corresponding to the peaks of interest. The minimum detection limits were reported to be 50 ppb for SULPH, 3 ppb for NASULPH, and 5 ppb for PYR. Within-day and day-to-day reproducibility are shown in Table 1. No significant degradation was detected for any of the compounds during storage for over six months.

The authors report that the ease of sample preparation, simultaneous quantitation, the small sample volume required, the low limit of detection of the compounds, and the short retention times all contribute to making the reported HPLC method suitable for routine analysis of Fansidar®. This method is presently being used in the author's laboratory for both routine clinical analyses and pharmacokinetic studies.

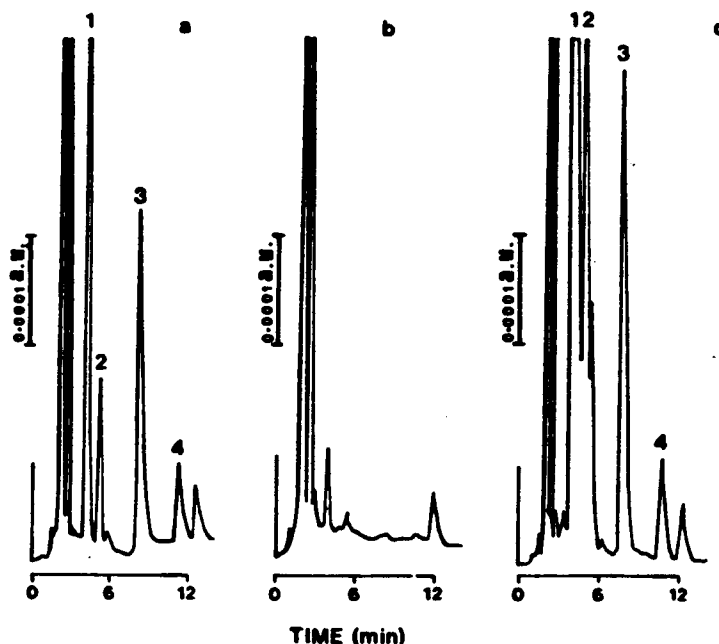


FIGURE 1. Chromatograms of (a) extracted spiked plasma sample containing SULPH, 0.45  $\mu\text{g}$  per 0.5 ml (1); NASULPH, 0.045  $\mu\text{g}$  per 0.5 ml (2); internal standard (quinine), 125 ng base (3) and PYR, 0.045  $\mu\text{g}$  per 0.5 ml (4); (b) extracted drug free plasma; and (c) extracted plasma sample obtained 168 h after Fansidar<sup>®</sup> administration to a healthy volunteer (concentrations found in this sample were: SULPH, 19.8  $\mu\text{g}$  per 0.5 ml (1); NASULPH, 0.678  $\mu\text{g}$  per 0.5 ml (2); quinine, 125 ng base (3); and PYR, 0.039  $\mu\text{g}$  per 0.5 ml (4).

FOR INVESTIGATIONAL USE ONLY.  
THE PERFORMANCE CHARACTERISTIC FOR THIS  
PROCEDURE HAS NOT BEEN ESTABLISHED.

TABLE I: PRECISION OF THE HPLC METHOD FOR SULPH, NASULPH AND PYR IN PLASMA (SPIKED SAMPLES)

The number of observations per compound per concentration = 5 in all cases

| Compound | Concentration<br>( $\mu\text{g}$ per 0.5 ml) | Coefficient of variation(%) |               |
|----------|--|-----------------------------|---------------|
|          |  | Within-day                  | Day-to-day    |
| SULPH    | 0.45   | 6.5                         | 13.7          |
|          | 1.82   | 4.1                         | 6.7           |
|          | 4.13   | 4.7                         | 4.8           |
|          | 22.73  | 2.9                         | 4.2           |
|          | Mean $\pm$ S.D.                              | 4.6 $\pm$ 1.5               | 7.4 $\pm$ 4.4 |
| NASULPH  | 0.045  | 7.7                         | 10.9          |
|          | 0.182  | 6.8                         | 8.3           |
|          | 0.413  | 3.1                         | 7.3           |
|          | 2.273  | 3.0                         | 5.5           |
|          | Mean $\pm$ S.D.                              | 5.2 $\pm$ 2.5               | 8.0 $\pm$ 2.3 |
| PYR      | 0.045  | 3.2                         | 4.4           |
|          | 0.182  | 3.9                         | 6.1           |
|          | 0.413  | 1.9                         | 4.6           |
|          | 2.273  | 2.2                         | 3.7           |
|          | Mean $\pm$ S.D.                              | 2.8 $\pm$ 0.9               | 4.7 $\pm$ 1.0 |