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Liquid Chromatography and Spectroscopic Method for Estimation of Sulphadoxine in Marketed Formulation

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ABSTRACT

A rapid and precise Reverse-Phase High-Performance Liquid Chromatographic method has been developed for the validation of Sulphadoxine in its pure form as well as in tablet dosage form. Chromatography was carried out on Apollo C18 (4.6 X 150 mm; 5µm) column using a mixture of ACN and water (15:85 v/v) as the mobile phase at a flow rate of 1.0 ml/min, the detection was carried out at 253 nm. The retention time of the sulphadoxine was 2.6±0.02 min respectively. The method produced linear responses in the concentration range of 5-25 µg/ml of sulphadoxine. The method precision for the determination of assay was below 2.0 %RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

INTRODUCTION

Sulfadoxine (4-Amino-N-(5,6-dimethoxy-4-pyrimidinyl) benzene sulfonamide) is a potential antimalarial drug. It is a sulfa drug, often used in combination with Pyrimethamine to treat malaria. Sulfa drugs or Sulfonamides are antimetabolites and compete with para-aminobenzoic acid (PABA) for incorporation into folic acid. It inhibits the enzyme *dihydropteroate synthetase*, which is an enzyme necessary in the conversion of PABA to folic acid, which is vital to the synthesis, repair, and methylation of DNA which is vital to cell growth in Plasmodium falciparum. With this vital nutrient lacking, the parasite has difficulty in reproducing[1] [2].

Literature survey reveals that certain chromatographic methods were reported for estimation of Sulphadoxine and single method is available for such estimation by RP-HPLC.

In view of the need for a suitable RP-HPLC method for routine analysis of Sulfadoxine in formulations, attempts were made to develop simple, precise and accurate analytical method for estimation of Sulphadoxine and extend it for their determination in the formulation. Validation is a necessary and important step in both framing and documenting the capabilities of the developed method[1][3].

MATERIALS AND METHODS

Equipment

Chromatography was carried out on HPLC system-WATERS, software: Empower 2, Alliance

2695 separation module. 486 PDA detector.

Chemical and reagents

Water, Methanol, and Acetonitrile (HPLC Grade) were obtained from Merck Specialities Pvt. Ltd. (Mumbai). Analytically pure samples of Sulfadoxine was obtained from Brown and Burk Pharmaceuticals Ltd., UK.

Preparation of standard solution

Accurately weighed 10 mg of Sulphadoxine working standard was taken into a 10 ml volumetric flask and about 7ml of Diluents were dissolved into it completely to make up the volume up to the mark (Stock solution). 0.15 ml of the above stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with Diluent to get a solution of 15 µg/ml concentration[1][2].

Preparation of sample solution

The average weight of Tablet was noted and it was crushed in a mortar by using a pestle. Then 10 mg equivalent weight of Sulphadoxine sample was taken into a 10 mL clean dry volumetric flask and about 7mL of Diluent was dissolved in it and volume made up to the mark. 0.15 ml of the above Sulphadoxine solution was taken into a 10 ml volumetric flask and diluted up to the mark with diluent[4][6].

Mobile Phase Preparation

150 ml (15%) of HPLC grade Acetonitrile and 850 ml of HPLC grade Water (85%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration[2].

RESULTS AND DISCUSSION

Optimized chromatographic conditions

Diluent: Mobile Phase

Mobile phase: ACN: Water (15:85)

Flow rate: 0.5 ml/min

Column: Hypersil ODS (C18), (250 X 4.6mm, 5µm)

Detector wavelength: 250 nm

Injection volume: 20µL

Runtime: 10 min

METHOD VALIDATION

The validation parameters for the proposed analytical method are elucidated as per the ICH guideline Q2R1.

Validation Parameters are as given below:

Linearity

Different solutions were prepared with concentrations, 0, 5, 10, 15, 20, 25 ppm of Sulfadoxine. Each solution was injected and linearity was evaluated by linear regression analysis.

The calibration curve of sulfadoxine is shown in Figure 1.

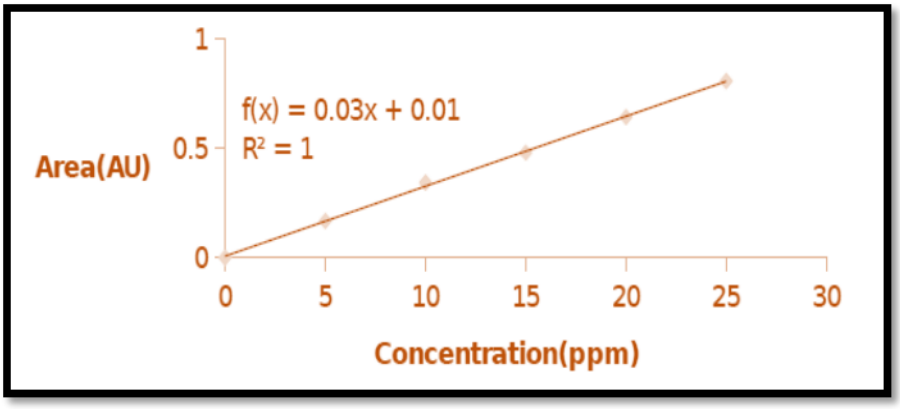

Figure 1: Calibration Curve

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of the standard to pre-analyzed sample preparation. For each concentration, three sets were prepared and injected. The results for accuracy are given in Table 1.

Table 1: Accuracy data and Percent recovery at different concentrations

%Concentration	Absorbance	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	0.247	7.5	7.5	100.2	100.6%
100%	0.483	15	15.1	100.1	
150%	0.725	22.5	22.9	101.6	

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions, recorded the absorbance and calculated % RSD. The results are mentioned in Table 2.

Table 2: Repeatability data and %RSD

S.no	Drug Name	Absorbance
1	Sulphadoxine	0.474
2	Sulphadoxine	0.475
3	Sulphadoxine	0.475
4	Sulphadoxine	0.474
5	Sulphadoxine	0.474
Mean		0.4744
Std. Dev.		0.000548
% RSD		0.115456

Robustness

The robustness was performed for the wavelength variations from 249 nm to 251 nm for Sulphadoxine. The method is robust by changing the wavelength condition. The data for robustness study is given in Table 3.

Table 3: Results for Robustness

The parameter used for sample analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
The actual Flow rate of 1.0 mL/min	631521	2.693	9544	1.12
Less Flow rate of 0.9 mL/min	631633	3.008	8474	1.2
More Flow rate of 1.1 mL/min	631047	2.303	8575	1.4
Less organic phase	631141	2.943	7285	1.17
More organic phase	634271	2.917	7264	1.2

The optimized chromatograms of the blank, sample, and standard are given in the figures 2, 3 and 4.

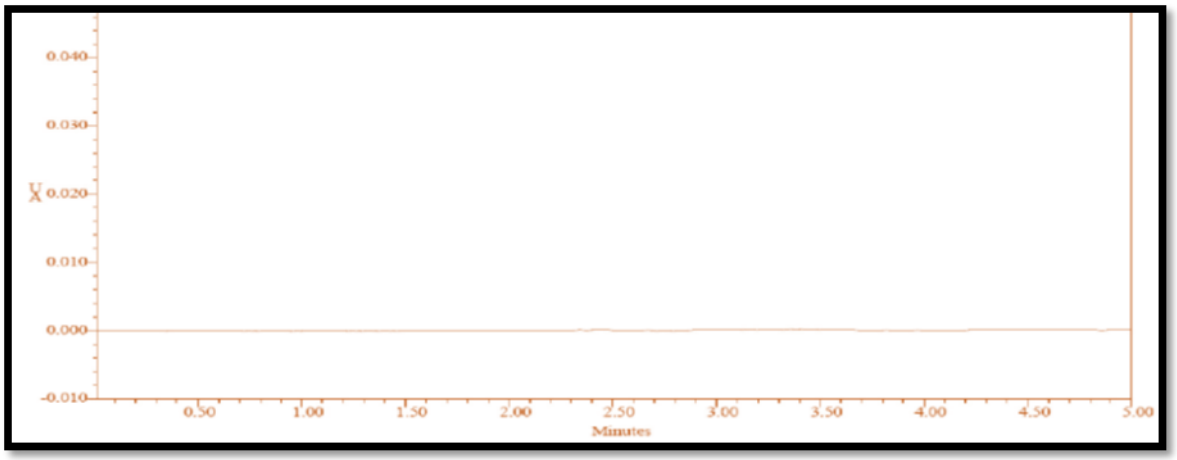

Figure 2: Chromatogram showing blank (mobile phase preparation)

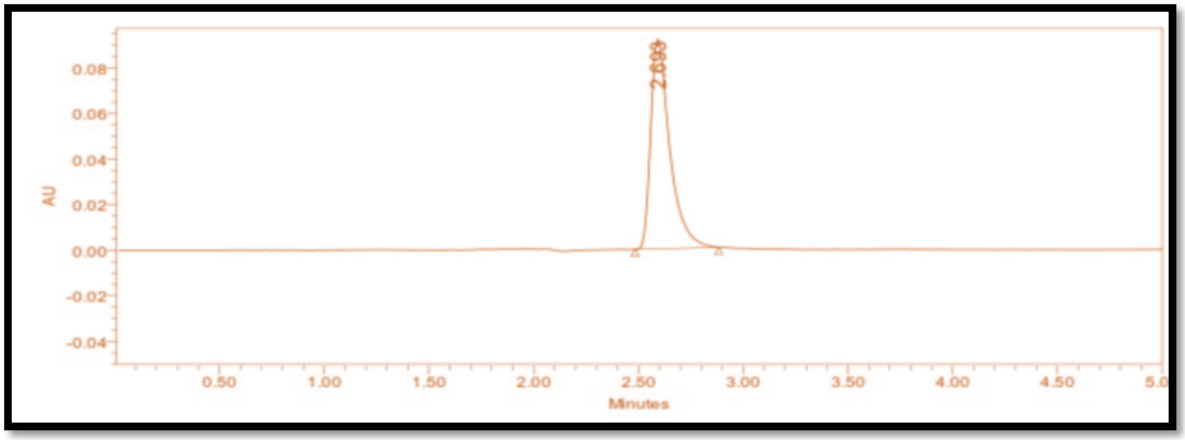

Figure 3: Optimized Chromatogram (Standard)

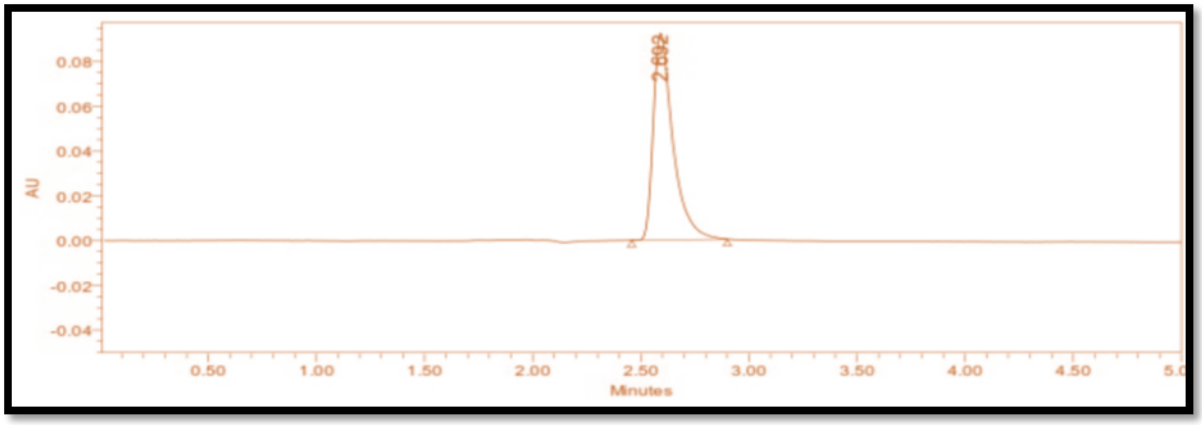

Figure 4: Optimized Chromatogram (Sample)

LOD and LOQ of Sulphadoxine

LOD and LOQ were calculated from the linear curve using formulae

LOD = 1.0 µg/ml

LOQ = 3.0 µg/ml

System Suitability Parameters

The data for system suitability parameters of the developed HPLC method are presented in Table 4.

Table 4: System suitability parameters

Sr. No.	Peak Name	RT	Area (µV X sec)	Height (µV)	USP Plate Count	USP Tailing
1	Sulphadoxine	2.693	631837	92251	9917	1.2
2	Sulphadoxine	2.692	631474	92274	9644	1.2
3	Sulphadoxine	2.692	631047	92291	9816	1.2
4	Sulphadoxine	2.691	631475	92183	9017	1.2
5	Sulphadoxine	2.694	631299	92291	9374	1.2
Mean			**631426.4**			
Std. Dev.			**288.7019**			
%RSD			**0.045722**			

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Sulphadoxine in bulk drug and pharmaceutical dosage forms. This method was simple since diluted samples were directly used without any preliminary chemical derivatization or purification steps. Sulphadoxine was freely soluble in ethanol, methanol and sparingly soluble in water. Acetonitrile: water was chosen as the mobile phase. The solvent system used in this method was economical.

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