

Development and Validation of a RP-HPLC Method for the Simultaneous Estimation of Sulfadoxine and Pyrimethamine in Combined Dosage Tablets

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ABSTRACT

A new simple, sensitive and reproducible RP-HPLC method for the simultaneous estimation of sulfadoxine and pyrimethamine in pharmaceutical formulations was developed and validated. The separation was carried out on Zorbax Eclipse Plus C18, 3.5 μm (4.6 \times 100 mm) column with acetonitrile: 20 mM sodium acetate buffer pH 6, 80:20% v/v as the mobile phase at the flow rate of 1 ml/min. The eluent detection was carried out using UV-Visible PDA detector at 224 nm. The retention time of sulfadoxine and pyrimethamine was 0.9 min and 1.2 min respectively. Linearity was observed for sulfadoxine and pyrimethamine in the concentration range of 60-200 $\mu\text{g/ml}$ and 3-10 $\mu\text{g/ml}$ respectively. The recovery of sulfadoxine and pyrimethamine was found to be 97.21-98.56% and 96.90-97.26% respectively.

Key words: RP-HPLC, Sulfadoxine, Pyrimethamine, Validation, Stress-induced stability.

INTRODUCTION

Sulfadoxine has been widely used in the treatment of various infections, such as cholera, leprosy, malaria and paracoccidiodomycosis. In combination with pyrimethamine, it is used in the treatment and prophylaxis of falciparum malaria resistant to other chemotherapeutic agents.¹ Pyrimethamine is a potent inhibitor of DHFR. The drug has shown to have a significantly higher affinity for binding to the DHFR of plasmodium than to the host enzyme (>1000 times in *Plasmodium berghei*) and, as a result, has been used to selectively treat plasmodium infections. The combination of pyrimethamine with a long acting sulfonamide, sulfadoxine, which blocks dihydrofolate synthesis by preventing incorporation of PABA into the dihydrofolate. The site of action of this combination is in the cytosol of plasmodium. The drug combination appears to have improved drug mediated disruption of folic acid in *Plasmodium sp.* The combination has been

used with quinine for prevention and treatment of chloroquine resistant malaria. The combination therapy has the added advantage of being inexpensive which is essential for successful therapy in developing countries.² Both the drugs are official in Indian Pharmacopoeia,³ British Pharmacopoeia⁴ and United States Pharmacopoeia.⁵ Chemically sulfadoxine is N¹-(5,6-dimethoxypyrimidin-4-yl)-sulphanilamide (Figure 1) and pyrimethamine is 5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine (Figure 2). On review of literature a few spectrophotometric,⁶⁻⁷ LC-MS⁸⁻¹⁰ and RP-HPLC¹¹⁻¹⁷ methods for the estimation of sulfadoxine and pyrimethamine in combination have been reported. As found from the literature sulfadoxine has been reported to be estimated in combination with other drugs by HPLC.¹⁸⁻¹⁹ Pyrimethamine has also been reported to be estimated by HPLC methods in tablets and in plasma.²⁰⁻²² The reported methods were reviewed. The first

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method¹² uses Kromasil C18, 5 μ (250 \times 4.6 mm) column with methanol, acetonitrile and 0.1% orthophosphoric acid in ratio 10:50:40, flow rate 1 ml/min at 223 nm, retention time were 4.67 min and 6.74 min for sulfadoxine and pyrimethamine respectively. The second method¹⁶ uses Xbridge C18, 5 μ (100 \times 4.6 mm) column with phosphate buffer: ACN 80:20 as mobile phase, flow rate 1ml/min at 223 nm, retention time was found to be 4.3 min for sulfadoxine and 6.3 min for pyrimethamine respectively. The third method¹⁷ uses ODS column with phosphate buffer of pH 3.2: ACN, 70:30 as mobile phase, flow rate 1 ml/min, retention time was found to be 4.1 min and 5.8 min for sulfadoxine and pyrimethamine respectively. For the reported methods phosphate buffers have been commonly used for the analysis of the sulfadoxine–pyrimethamine combination and retention times have been consistently found between 4 to 6 min. The objective of the present work was to develop a simultaneous method for the determination of sulfadoxine and pyrimethamine in combination with volatile buffer like acetate buffer for possible LCMS analysis, validate the method and apply the same for estimation of marketed formulation.

MATERIAL AND METHODS

Equipment

HPLC of Agilent 1260 make equipped with autosampler and auto injector with PDA detector and Double beam UV/VIS spectrophotometer of Lab India[®] make of UV 3000+ Analytical Tech model were used for the study. Sulfadoxine and pyrimethamine reference standards were obtained from R L FineChem, Bangalore. Combined tablet dosage formulations as Falcigo-SP Kit from Aeon formulations Pvt. Ltd and Larinate-100 Kit from Ipca laboratories Ltd. containing 500 mg of sulfadoxine and 25 mg of pyrimethamine per tablet were purchased from the local market. HPLC grade acetonitrile and water was procured from Merck Specialities Private Limited, Mumbai. Sodium acetate and glacial acetic acid (AR grade) was obtained from Loba Chemie Pvt. Ltd. and Molychem, Mumbai respectively.

Chromatographic conditions

Zorbax Eclipse Plus C18, 3.5 μ m (4.6 \times 100 mm) column was used for the separation. Acetonitrile: 20 mM sodium acetate buffer pH 6, 80:20% v/v was delivered at a flow rate of 1 ml/min and detection carried out at 224 nm. The injection volume was 5 μ l and the analysis was performed at ambient temperature.

Standard stock solution

Std stock solution of sulfadoxine and pyrimethamine were prepared separately in mobile phase of concentration 1000 μ g/ml and 100 μ g/ml respectively. Working std solution was prepared by mixing 1.3 ml of sulfadoxine and 6.5 ml of pyrimethamine std stock solutions in 10 ml std flask, diluted with the mobile phase to the mark. The retention time of sulfadoxine and pyrimethamine were found to be 0.9 min and 1.2 min, respectively. Calibration curves were constructed by plotting the average peak areas against the respective concentrations and found to be linear in the range of 60-200 μ g/ml and 3-10 μ g/ml for sulfadoxine and pyrimethamine respectively, r^2 being 0.999 for both the drugs.

Analysis of sulfadoxine and pyrimethamine in marketed tablets

Twenty tablets were accurately weighed and finely powdered. An amount of tablet powder equivalent to 100 mg of sulfadoxine was transferred into a 100 ml std flask. About 50 ml of mobile phase was added to the flask and sonicated for 15 min to disperse the contents completely. The volume was made up to the mark with the mobile phase and filtered using Whatman filter paper (no. 45) to obtain sample stock solution. From this 1.3 ml was transferred into a 10 ml calibrated std flask and volume made up with the mobile phase to obtain working sample solution.

VALIDATION

The method was validated for linearity, precision, specificity, accuracy, ruggedness and robustness as per the ICH guidelines.²³ The linearity for sulfadoxine and pyrimethamine was found to be in the range of 60-200 μ g/ml and 3-10 μ g/ml respectively. Accuracy was determined at three different levels of 80%, 100% and 120% of the target concentration of the active ingredient, by adding a known amount of each std to previously analysed tablet sample. Precision was studied to determine intraday variation by performing six replicate assays of the tablet sample. The % RSD was calculated for intraday precision. The LOD and LOQ were calculated from the standard calibration curves. The residual standard deviation of the regression line or the standard deviation of the y-intercepts of regression lines was used as the standard deviation. LOD and LOQ were calculated with equations $LOD=3.3\sigma/S$ and $LOQ=10\sigma/S$; where, σ is the Standard deviation of the response and S is the slope of the calibration curve. Robustness of the method was determined by making slight changes in the operating conditions viz.

Table 1: Validation and System suitability Method of the Proposed Method

Parameters	Sulfadoxine	Pyrimethamine
Theoretical plates	2261	3020
Resolution	3.2	
Asymmetric factor	1.19	1.03
Retention Time (min)	0.9	1.2
Linearity range ($\mu\text{g/ml}$)	60-200	3-10
Regression Equation $y=mx+c$	$y=1263660.624x + 3916998.722$	$y=2742921.802x + 1489716.623$
Slope (m)	1263660.624	2742921.802
Intercept (c)	3916998.722	1489716.623
Correlation coefficient	0.999	0.999
Intra day(n 6) % RSD	0.28	0.33
LOD ($\mu\text{g/ml}$)	5.93	0.33
LOQ ($\mu\text{g/ml}$)	17.99	1.018

*Average of six determination, y is the area under curve and x is the concentration, RSD is the relative standard deviation, LOD- Limit of detection, LOQ- Limit of Quantitation.

Table 2: Robustness Study

Parameters	Proposed	Variation	Sulfadoxine	Pyrimethamine
			% Assay	
Flow rate	1.0 ml/min	0.8 ml/min	100.74	99.74
		1.2 ml/min	96.05	97.71
Mobile phase composition	80:20%	75:25%	97.45	93.93
		85:15%	96.06	98.57
pH	6.0	5.8	102.1	101.5
		6.2	95.87	96.1

Table 3: % Recovery for Sulfadoxine and Pyrimethamine

Sulfadoxine				Pyrimethamine		
Level of std addition(%)	Amount present in sample ($\mu\text{g/ml}$)	Amount of std added ($\mu\text{g/ml}$)	% Recovery*	Amount present in sample ($\mu\text{g/ml}$)	Amount of std added ($\mu\text{g/ml}$)	% Recovery*
80	130	104.0	98.563	6.5	5.2	97.263
100		130.0	97.609		6.5	96.900
120		156.0	97.208		7.8	97.264

*Average of three determinations.

flow rate ± 0.2 ml, change in the organic phase ratio by $\pm 5\%$ and pH by ± 0.2 units. Specificity was determined using a placebo of commonly used tablet excipients. The solution stability was determined by performing the analysis up to 12 hr with the working solutions. The mixed standard solutions of sulfadoxine and pyrimethamine were then subjected to stress studies under acid and base hydrolysis conditions by refluxing with 0.1 M hydrochloric acid and 0.1 M sodium hydroxide solutions for 3 hr separately. Also both the standard substances as solids was subjected to high temperature of 80°C for

3 hr and exposure to UV radiation for 1 hr. The resulting solutions were assayed by the validated process.

RESULTS AND DISCUSSION

The aim of the present study was to develop a rapid and precise RP-HPLC method for the estimation of sulfadoxine and pyrimethamine in combined dosage form, with volatile buffers and validate in accordance with ICH guidelines. Exploratory trials were performed in order to select adequate and optimum conditions to

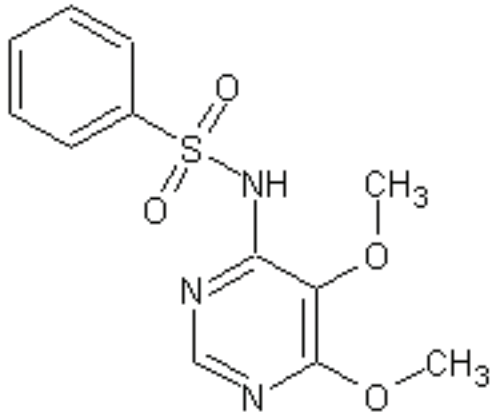


Figure 1: Chemical Structure of Sulfadoxine.

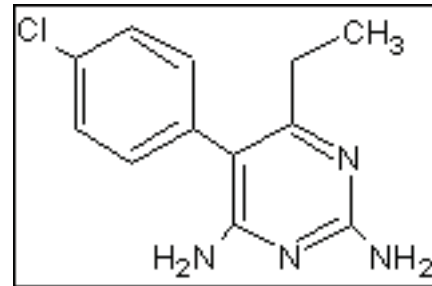


Figure 2: Chemical Structure of Pyrimethamine.

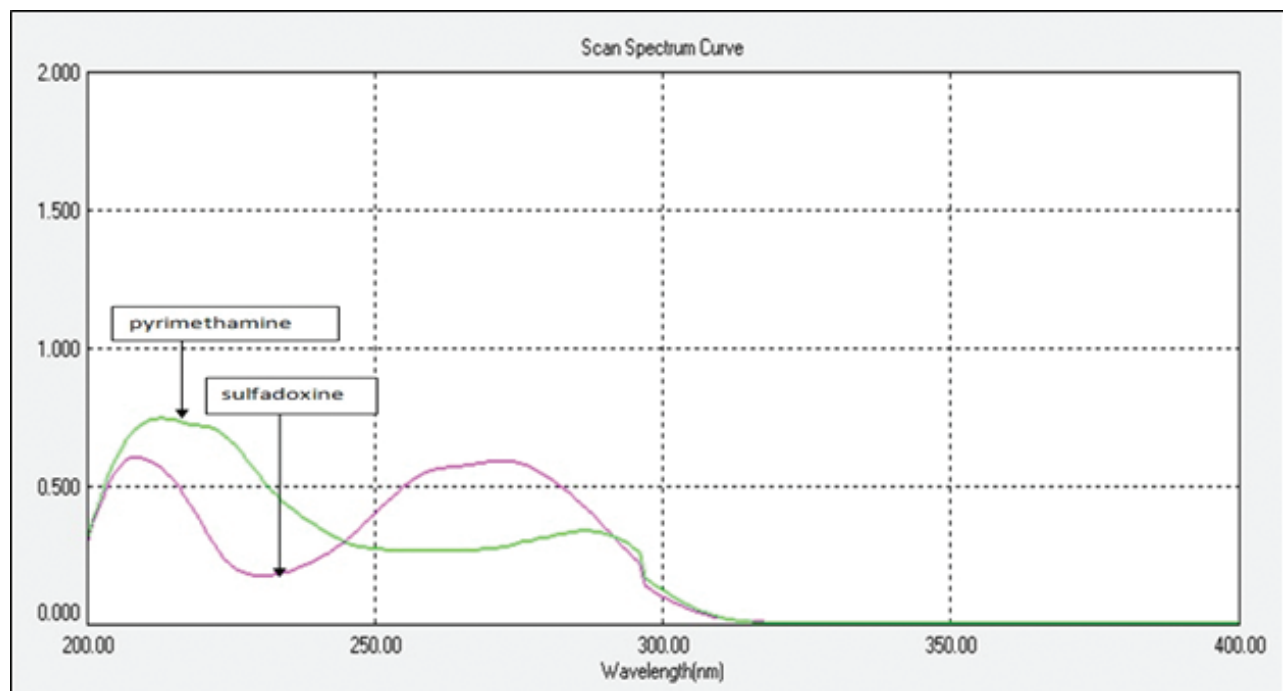


Figure 3: Overlay UV spectrum of sulfadoxine and pyrimethamine in optimized mobile phase.

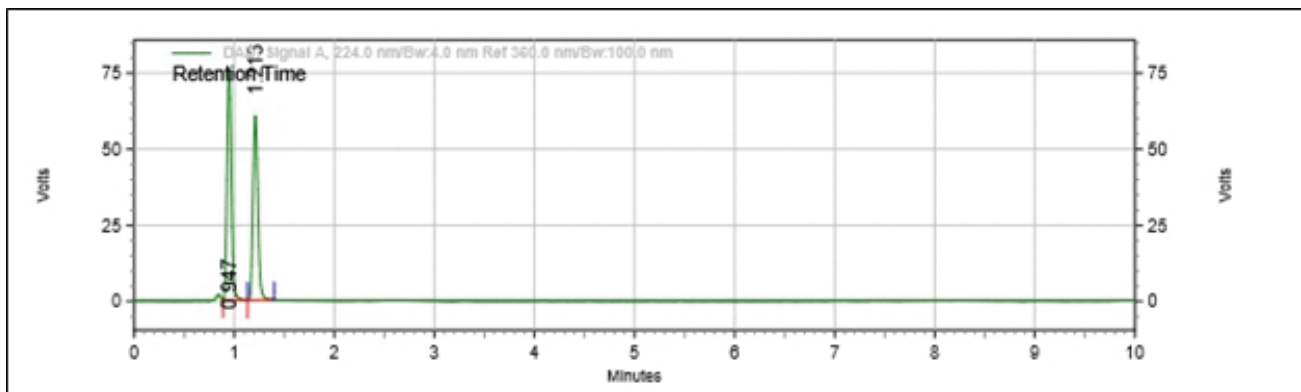


Figure 4: Typical chromatogram showing separation of sulfadoxine and pyrimethamine in combined dosage dosage form.

develop an effective method. UV overlain spectra of both sulfadoxine and pyrimethamine showed that both the drugs absorb appreciably at 224 nm (Figure 3). As concentration of sulfadoxine is much higher than pyrimethamine in marketed dosage form in the ratio of 20:1, a wavelength suitable for detection of both in such disproportionate ratio was possible at 224 nm, wherein absorbance was higher for pyrimethamine in comparison to sulfadoxine by significant folds for solutions of matching concentration. The mobile phase containing acetonitrile: 20 mM sodium acetate buffer pH 6.0, 80:20% with a flow rate of 1 ml/min was selected for analysis after preliminary studies. The retention time of sulfadoxine and pyrimethamine was 0.9 min and 1.2 min respectively. Resolution of the method was found to be 3.2, which is highly satisfactory and indicates good separation of compound peaks.

Typical chromatogram of sulfadoxine and pyrimethamine is shown in Figure 4. The system suitability tests were carried out and parameters are summarized in Table 1. The tailing factor was calculated statistically and found to be 1.19 and 1.03 for sulfadoxine and pyrimethamine respectively. The standard calibration curves for the drug mixtures were plotted and the linearity range obtained was 60-200 µg/ml for sulfadoxine and 3-10 µg/ml for pyrimethamine. Linearity coefficient and percentage curve fitting were well within the limits as found from the value of 0.999 and 0.999 for sulfadoxine and pyrimethamine respectively. The % RSD of assay was found to be within limit of 2% (Table 1), indicating high degree of precision and reproducibility. There was no visible peak in the retention time upto 10 min indicating high degree of specificity of the proposed method. Accuracy was determined through recovery study of the drug at three levels ranging from 80-120% and was found to be well within the acceptance limit indicating no interferences of the drug with each other nor with the excipients present in the formulation (Table 3).

The robustness of the method (Table 2) was determined by carrying out the assay by slightly varying the mobile phase ratio, pH and flow rate and the percentage recovery was found to be within limits (Table 3). The detection

limit was found to be 5.93 µg/ml for sulfadoxine and 0.33 µg/ml for pyrimethamine while the quantitation limit was found to be 17.99 µg/ml for sulfadoxine and 1.01 µg/ml for pyrimethamine which suggests that the amount of both the compounds can be estimated accurately.

It was observed that the retention time and peak area of all the working standard and sample solutions remained unchanged and no significant degradation observed up to 12 hr. For stress stability studies, the peak positions remained constant and peak area response did not alter by more than 2% indicating stability of sulfadoxine and pyrimethamine under all the stress conditions. So the proposed method is specific, reproducible and accurate for the analysis of sulfadoxine and pyrimethamine in combined dosage form.

CONCLUSION

A new RP-HPLC method for the simultaneous estimation of sulfadoxine and pyrimethamine in pharmaceutical formulations was developed using simple acetate based mobile phase on a C-18 ODS column of 10 cm length. Separation was complete with shorter analysis time compared to reported methods with 25 cm ODS column with non-volatile phosphate buffers. The method was accurate and found to be simple, specific, reproducible, precise and robust.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATIONS USED

Std: Standard

ICH: International Conference on Harmonisation.

SUMMARY

- RP-HPLC method for simultaneous analysis of sulfadoxine and pyrimethamine in tablet formulation was developed and validated on Zorbax Eclipse plus C 18, 3.5 µm (4.6 × 100 mm) column.
- Mobile phase composition acetonitrile: 20 mM sodium acetate buffer pH 6 in the ratio 80:20% v/v at flow rate of 1 ml/min provided low net retention times with good resolution.

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