INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.10 | Issue 3 | Jul - Sep-2021
Journal Home page: www.ijpar.com

Review Study *Open Access*

Analytical method development and validation for the simultaneous estimation of sulfadiazine and pyrimethamine by rp-hplc method

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ABSTRACT

Background

Estimation of Sulfadiazine and Pyrimethamine is a combination drug of choice used to treat malaria who are living in or will be travelling to an area where there is chance of getting malaria.

Objective

The regimen above is in short supply and to evaluate efficacy of twice weekly maintenance therapy to prevent recurrent toxoplasmic encephalitis in patients with Acquired Immnuno deficiency syndrome.

Materials and methods

A HPLC (Alliance,Water2695) with UV/VIS Detector/PDA detector, UV (lab India,UV 3000 series) and Agilent C18 250mm × 4.6mm × 5µm column was used. A new method was established for simultaneous estimation of Sulfadiazine and Pyrimethamine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Sulfadiazine and Pyrimethamine by using Zodiac sil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid),detection wavelength was 240nm.

Results

The results were in good agreement with those obtained with official HPLC with absorption maximum of 240 nm by preparing mobile phase 70:30 methanol **:** phosphate buffer with flow rate 1 ml/min and it run for 10 minutes by selecting column Zodiac silca RP C18 4.5×100 mm 3.0 µm of ambient temperature .All the results obtained with good precise ,accurate and robustness as per ICH guidelines.

Conclusion
It can be concluded that the proposed RPHPLC method is accurate, precise, sensitive, specific, robust and reproducible for the simultaneous analysis of Sulfadiazine and Pyrimethamine with less tailing factor and is also economical. Zodiac sil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid),detection wavelength was 240nm.

Keywords: Zodiac silica C18 column, Sulfadiazine and Pyrimethamine, RP-HPLC

INTRODUCTION

Sulfadiazine is 4-amino-N-(pyrimidin-2-yl)benzene-1-sulfonamide and belongs to category anti-Infective agents.Pyrimethamine is 5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine and belongs to category Anti malarial and anti Infective agents. It acts by Inhibiting bacterial enzyme dihydropteroate synthetase which is essential for folic acid synthesis and used to treat infections of second and third degree burns.Pyrimethamine it acts by selectively inhibits the plasmodial form of Dihydrofolate reductase it stopping this parasite from reproducing once it is in the blood stream.

Chemicals and Reagents

Sulfadiazine and Pyrimethamine were obtained as gift samples from AURUBINDO labs Pvt. ltd, Hyderabad. We used HPLC grade acetonitrile, water and GR grade KH_2PO_4 and ortho phosphoric acid.

Instrumentation

A HPLC (Alliance,Water2695) with UV/VIS Detector/PDA detector, UV (lab India,UV 3000 series) and Agilent C18 250mm × 4.6mm × 5µm column was used. The HPLC system was equipped with Empower software for data processing.

Chromatographic Condition

The mobile phase containing mobile phase 70:30 methanol**:** phosphate buffer was found to resolve Sulfadiazine and Pyrimethamine. Ortho phosphoric acid was used for pH adjustment of buffer to 4 .0. The mobile phase was filtered through 0.45 nylon filter and then ultrasonicated for 30 min. The flow rate was set to 1.0ml/min. The drug shows good absorbance at 240nm, which was selected as wavelength for further analysis.

Preparation of Mobile Phase

Mix a mixture of above methanol 700 ml (70%) and 300 ml of buffer (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Preparation of Sample solution

Accurately weigh and transfer 8 mg of Sulfadiazine and 12.5 mg Pyrimethamine Tablet powder into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of Sulfadiazine &Pyrimethamine the above stock solution into a10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Standard stock solutions

Accurately weighed 8mg of Sulfadiazine and 12.5 mg Pyrimethamine transferred10ml and volumetric flasks, 3/4th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (5000µg/ml of Sulfadiazine and Pyrimethamine)

Preparation of Standard working solutions (100% solution)

1ml of Sulfadiazine and Pyrimethamine from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (500µg/ml of Sulfadiazine and Pyrimethamine)

Preparation of Sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters.(5000 µg/ml of Sulfadiazine and Pyrimethamine).

Preparation of Sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (500µg/ml of Sulfadiazine and Pyrimethamine)

Preparation of buffer

0.1%OPA Buffer: 1ml of Perchloric acid was diluted to 1000ml with HPLC grade water.

Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Sulfadiazine and Pyrimethamine was obtained and the isobestic point of Sulfadiazine and Pyrimethamine showed absorbance's maxima at 240 nm. Chromatographic method development were optimized by various parameters both in API and pharmaceutical dosage form in Figure 1.

The Optimised Chromatographic conditions by preparing mobile phase 70:30 methanol**:** phosphate buffer with flow rate 1 ml/min and it run for 10 minutes by selecting column Zodiac silca RP C18 4.5×100 mm 3.0 µm of ambient temperature.

The retention time of Sulfadiazine and Pyrimethamine was found to be 2.170 mins and 7.280 mins respectively. Resolution was 8.67.The % purity Sulfadiazine and Pyrimethamine in pharmaceutical dosage form was found to be 99.1 and 98.2% respectively was shown in Table no 1 & Figure no 2 .

VALIDATION REPORT

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak.

LINEARITY

The linearity study was performed for the concentration of 25 ppm to 150 for Pyrimethamine and 16ppm to 80ppm for Sulfadiazine was shown in Table no 2

ACCURACY

The accuracy study was performed for 50%, 100% and 150 % for Sulfadiazine and Pyrimethamine. The % recovery was found to be 101.4% and 101.7% was shown in Table no 3.

Precision (Repeatbility)

The precision study was performed for five injections of Sulfadiazine and Pyrimethamine. Each standard injection was injected into chromatographic system.The intermediate precision study was performed for five injections was shown in Table no 4.

LOD and LOQ

The LOD was performed for Sulfadiazine and Pyrimethamine was found to be 2.17and 0.0372 respectively. The LOQ was performed for Sulfadiazine and Pyrimethamine was found to be 6.60 and 0.112 respectively was shown in Fig No 3.

Robustness

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Sulfadiazine and Pyrimethamine it can be concluded that the variation in flow rate affected the method significantly was shown in Table no 5.

Degradation studies

Oxidation

To 1 ml of stock solution ofSulfadiazine and Pyrimethamine, 1 ml of 20% hydrogen peroxide (H2O2) wasadded separately. The solutions were kept for 30 min at 60^0c. For HPLC study the resultant solution to obtain 500µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of sample was shown in Table no.6

Acid Degradation Studies

To 1 ml of stock solution Sulfadiazine and Pyrimethamine, 1 ml of 2N was added to resultant Hydrochloric acid. The resultant solution was diluted to obtain 500µg/ml solution 10 microllitre and subjected to the stability of the sample.

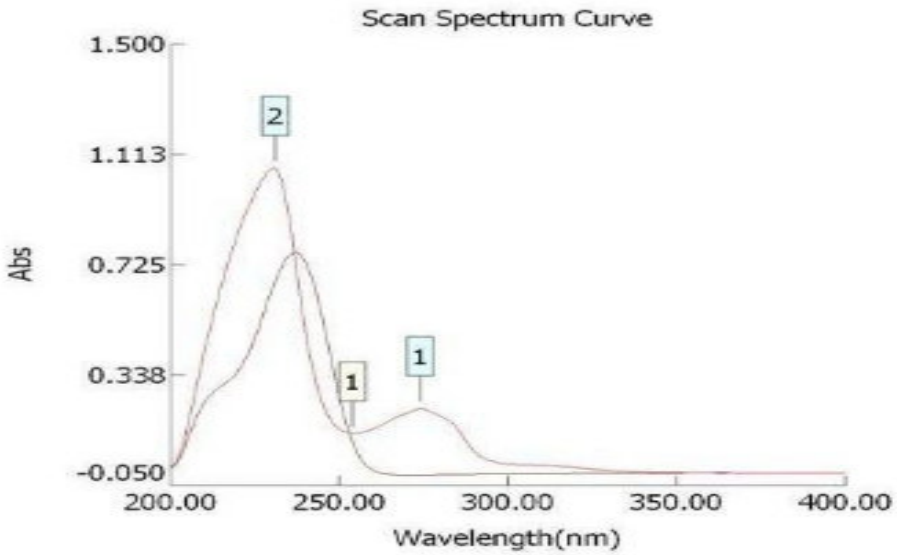

Fig No 1:Spectrum showing overlapping spectrum of Sulfadiazine and Pyrimethamine

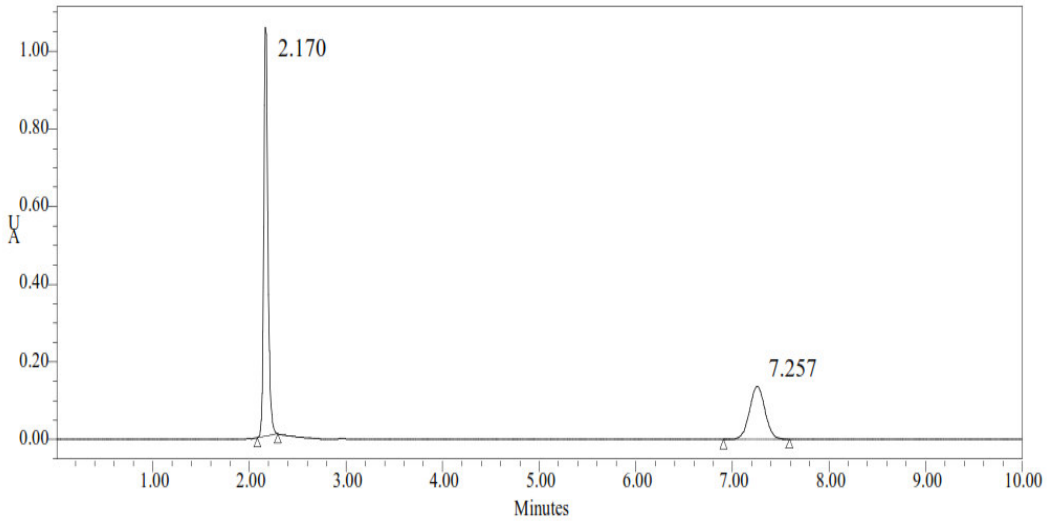

Fig No 2: The retention time of Sulfadiazine and Pyrimethamine was found to be 2.170 mins and 7.257 mins respectively.

Table no 1: The % purity Sulfadiazine and Pyrimethamine in pharmaceutical dosage form was found to be 99.1 and 98.2% respectively.

S. No	Name of compound	Label claim(mg)	Amount taken(mg)	%purity
1	Sulfadiazine	8	58.9	99.1
2	Pyrimethamine	12.5	58.9	98.2

Table no 2: Linearity Results for Suifadizine and Pyrimethamine

S.No	Linearity Level	Concentration Sulfadiazine	Concentration Pyrimethamine	Area (SULF)	Area(PYR)
1	I	16ppm	25ppm	1027461	2201022

2	II	32ppm	50ppm	1233566	2585033
3	III	48ppm	75ppm	1437030	2996553
4	IV	64ppm	100ppm	1644336	3446224
5	V	80ppm	125ppm	1880590	3897922
Correlation Coefficient				**0.999**	

Table no 3: Accuracy results of Sulfadiazine and Pyrimethamine

%Concentration (at specification Level)	Area SULF	Area PYR	Amount Added (mg)		Amount Found (mg)		% Recovery		Mean Recovery SULF & PYR
			SULF	PYR	SULF	PYR			
50%	765624	1726242	4.25	7.05	4.30	7.1	101.2%	101.9%	101.4%
100%	1508055	3187170	8.25	13.1	8.48	13.2	01.5%	101.3%	
150%	2204983	4521881	12.2	18.5	12.39	18.8	101.6%	101.8%	101.7%

Table no :4 The Method precision study was performed for the %RSD of Sulfadiazine and Pyrimethamine was found to be 0.5 and 0.8

Injection	Area(SULF)	Area (PYR)
Injection-1	1475698	3045768
Injection-2	1461561	3030853
Injection-3	1481379	3063519
Injection-4	1467049	3065127
Injection-5	1472628	3099001
Average	1471663	3060854
Standard Deviation	7664.08	25535.28
%RSD	0.52	0.83

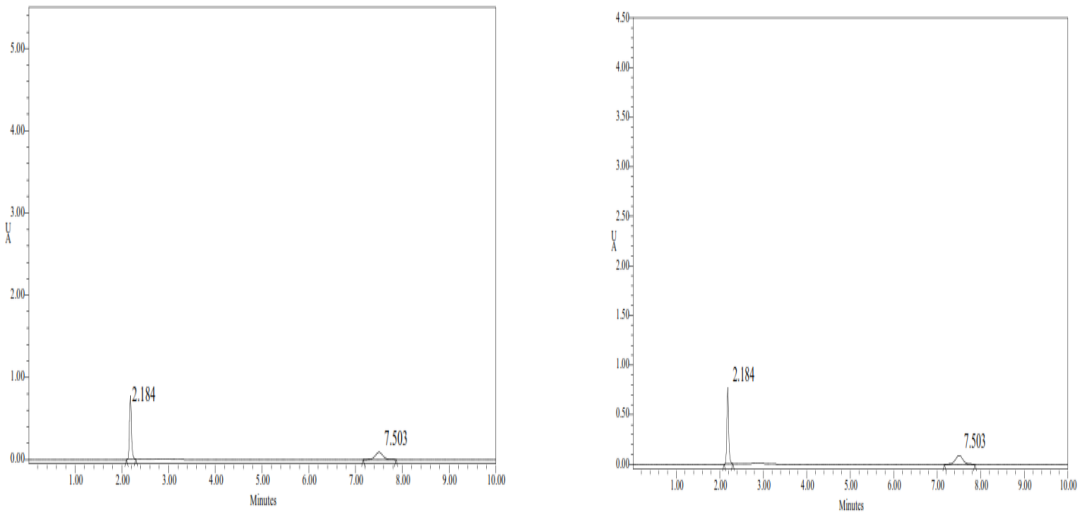

Fig No 3:The LOD was performed for Sulfadiazine and Pyrimethamine was found to be 2.17and 0.0372 respectively and LOQ was found to be 6.60 and 0.112 respectively.

Table no 5:Robustness results for Sulfadiazine and Pyrimethamine

S.No.	Change in Organic Composition in the Mobile Phase	System Suitability Results			
		USP Plate Count	USP Plate Count	USP Tailing	USP Tailing
1	10% less	6953.5	7079.0	1.0	1.0
2	*Actual	10026.7	12458.5	1.2.0	1.0
3	10% more	6048.5	228.56	1. 1.0	1.0

Table no.6: Chromatogram showing Observation: Degradation data of Sulfadiazine and Pyrimethamine

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.01	0.295	0.345
2	Alkali	3.96	0.325	0.360
3	Oxidation	0.523	0.873	0.577
4	Thermal	0.51	0.193	0.328
5	UV	0.90	0.430	0.535
6	Water	0.07	0.264	0.331

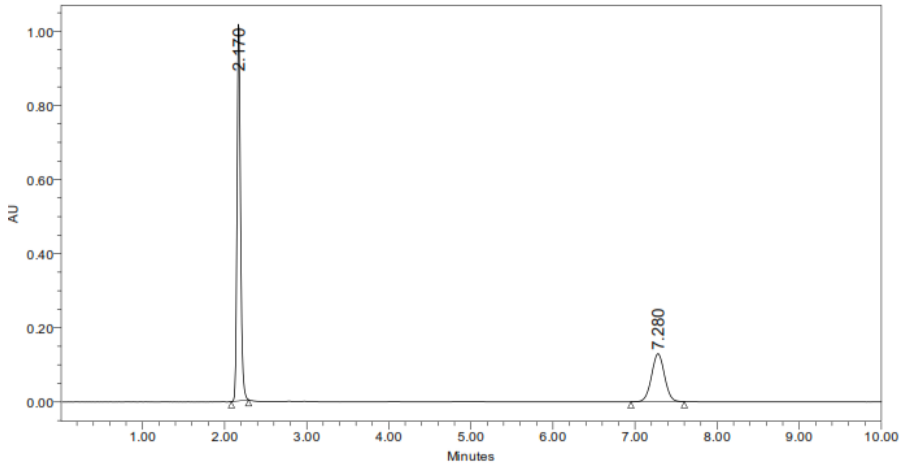

Fig no.4 Chromatogram showing Observation: Peroxide degradation Chromatogram of Sulfadiazine and Pyrimethamine.

CONCLUSION

It can be concluded that the proposed RPHPLC method is accurate, precise, sensitive, specific, robust and reproducible for the simultaneous analysis of Sulfadiazine and Pyrimethamine with less tailing factor and is also economical. Zodiac sil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid),detection wavelength was 240nm.

CONSENT AND ETHICAL APPROVAL

It is not applicable

ACKNOWLEDGEMENTS

We gratefully acknowledge ABIPER and RGUHS university for providing all needed things to prepare this manuscript

COMPETING INTEREST

Authors have declared that no completing interests exists

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