International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

R E S E A R C H A R T I C L E

Analytical Method Development and Validation for Simultaneous Estimation of Pyrimethamine and Sulphadoxine in Pharmaceutical Dosage form by RP-HPLC

N. Kalpana Devi*[1], Tirupathi Rao Velamala[2], D.S.S.N.Neelima[3]

[1,2] Teegala Krishna Reddy College of Pharmacy, Hyderabad, Telangana State
[3] Vikas Institite of Pharmaceutical Sciences, Rajahmundary

A B S T R A C T

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Sulphadoxine and Pyrimethamine, in its pure form as well as in tablet dosage form. Chromatography was carried out on an XBridge C18 (4.6×250mm) 5µ column using a mixture of Phosphate Buffer: Acetonitrile (70:30) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 224nm. The retention time of the Pyrimethamine and Sulphadoxine was 2.8, 3.4±0.02min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Pyrimethamine and 100-750µg/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.

Keywords: Pyrimethamine, Sulphadoxine, RP-HPLC, validation.

A R T I C L E I N F O

Corresponding Author
N. Kalpana Devi
Department of Pharmaceutical Analysis
Teegala Krishna Reddy College of Pharmacy,
Hyderabad, Telangana State.
MS-ID: IJCTPR3669

PAPER QR-CODE

Article History: Received 11April 2018, **Accepted** 27 May 2018, **Available Online**15 July 2018

Citation: N. Kalpana Devi, *et al.Analytical Method Development and Validation for Simultaneous Estimation of Pyrimethamine and Sulphadoxine in Pharmaceutical Dosage form by RP-HPLC. Int. J. Curnt. Tren. Pharm, Res., Res*., 2018, 6(4): 97-102.

C O N T E N T S

1. Introduction

Pharmaceutical analysis1 is a branch of Analytical chemistry that involves a series of process for identification, determination, quantification and purification of a substance and also separation of the components of the solution or a mixture, or determination of structure of chemical compounds. The substance may be a single

compound or a mixture of compounds and it may be in any of the dosage form. The drug substance used as pharmaceuticals are from any of the source like animals, plants, microorganisms, minerals and various synthetic products. It is of Synthetic origin and belongs to Pyrimidine. It belongs to Dihydrofolate reductase inhibitor pharmacological group on the basis of mechanism of action. Chemically Pyrimethamineis 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine and sulphadoxine is 4-amino-N-(5,6-dimethoxy-4- pyrimidinyl) benzenesulfonamide. Sulphadoxine also known as Sulformethoxine. It is of synthetic origin and belongs to Sulphonamide. It belongs to Dihydropteroate synthetase inhibitor pharmacological group on the basis of mechanism of action [1,2].

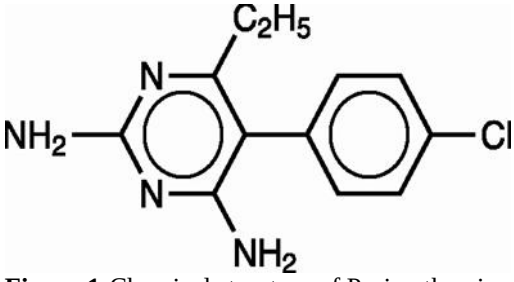

Figure 1: Chemical structure of Pyrimethamine

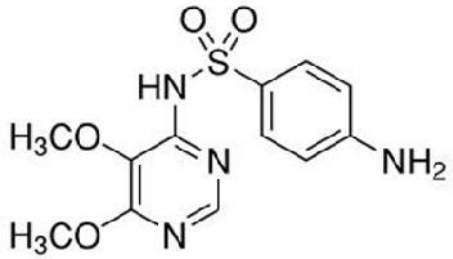

Figure 2: Chemical structure of Sulphadoxine

2. Materials and Methods

Chromatographic conditions

A perominence isocratic HPLC system (waters 2695 HPLC with auto sampler and PDA Detector) column XBridge C18 (4.6 x 250mm, 5µm). A 10µL Rheodyne injection syringe was used for sample injection. HPLC grade Water and Acetonitrile were used for the preparing the mobile phase. A freshly prepared Phosphate Buffer : Acetonitrile (70:30) was used as the mobile phase. The solvents was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1mL/min., column temperature was maintained at room temperature and the detection of the drug was carried out at 244nm.

Preparation of mobile phase

Mix a mixture of above Phosphate Buffer 700mL (70%) and 300mL of Acetonitrile HPLC (30%) and degas in ultrasonic water bath for 10minutes. Filter through 0.45µ filter under vacuum filtration.

Diluent preparation:

Mobile phase as diluent.

Standard solution preparation:

Standard stock solution of Pyrimethamine (500µg/ml): Accurately weighed 25mg of Pyrimethamine was taken in a clean, dry 50ml volumetric flask, added with sufficient volume of diluent and sonicated for 5min. Then the solution was filtered and volume was made up to 50ml with the diluent.

Standard stock solution of Sulphadoxine (10000µg/ml):

Accurately weighed 500 mg of Sulphadoxine was taken in a clean, dry 50 ml volumetric flask, added with sufficient volume of diluent and sonicated for 5min. Then the solution was filtered and volume was made up to 50ml with the diluent.

Procedure:

0.5 ml of standard stock solution of Pyrimethamine (500µg/ml) and 0.5 ml of standard stock solution of Sulphadoxine (10000µg/ml) were transferred into a 10ml volumetric flask and the volume was made up with diluent. The resulted solution was sonicated for 10 min and injected into the HPLC system .The retention time, peak area, USP plate count and peak resolution were observed. The results were shown in Table No.8 and the chromatogram was presented in Fig 3.

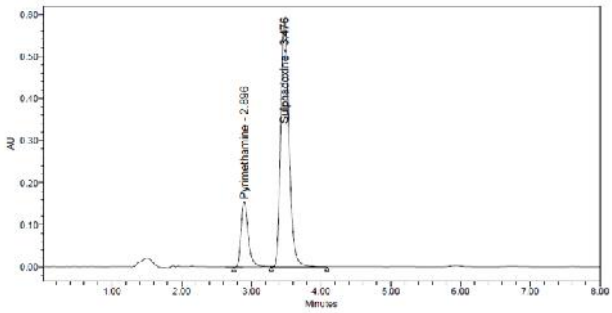

Figure 3: Chromatogram of Pyrimethamine and Sulphadoxine at 224nm

Sample solution preparation:

5 tablets were weighed and average weight was determined and powdered. An amount of powder equivalent to average weight of 5 tablets (equivalent to 25mg of Pyrimethamine and 500mg of Sulphadoxine) was weighed and transferred into a 100 ml volumetric flask. 60ml of diluent was added and sonicated for 25 min to ensure complete dissolution. Then the solution was filtered and further the volume was made up with diluent. From this solution, 0.2ml was taken into a 10 ml volumetric flask and made up the volume with diluent.

Sample solution preparation:

5 tablets were weighed and average weight was determined and powdered. An amount of powder equivalent to average weight of 5 tablets (equivalent to 25mg of Pyrimethamine and 500mg of Sulphadoxine) was weighed and transferred into a 100 ml volumetric flask. 60ml of diluent was added and sonicated for 25 min to ensure complete dissolution. Then the solution was filtered and further the volume was made up with diluent. From this solution, 0.2ml was taken into a 10 ml volumetric flask and made up the volume with diluent.

Method validation[3-12]

Linearity:

The linearity of the method was demonstrated over the concentration range of 5-37.5ppm of the Pyrimethamine target concentration and 100-750ppm of Sulphadoxine. Different concentrations of the pure drug were injected into the chromatographic system. Calibration curve of Pyrimethamine and Sulphadoxine was constructed by plotting peak area versus applied concentration of Pyrimethamine and Sulphadoxine. A typical chromatogram is shown in Fig 1. The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range & it has shown in fig: 2 and 3. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in Table: 1&2 and their calibration parameters were shown in Table: 3&4.

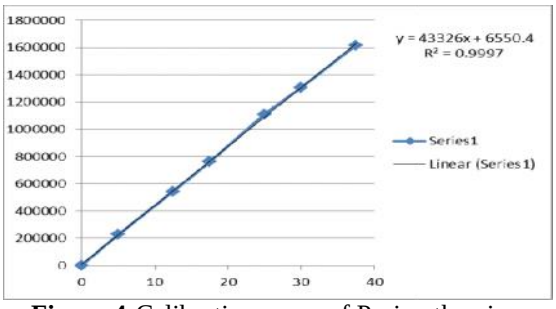

Figure 4: Calibration curve of Pyrimethamine

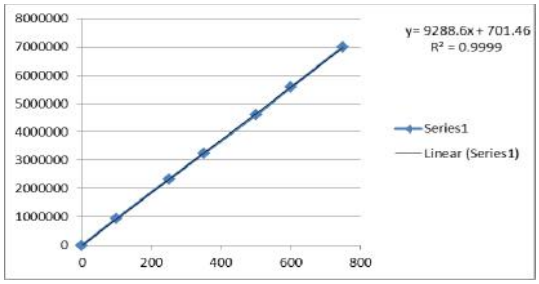

Figure 5: Calibration curve of Sulphadoxine

Precision method

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution was made and the response factor of drug peak and% RSD were calculated and present in Table 5&6. The chromatogram was shown in Fig 4. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drug peak and %RSD were calculated shown in Table5&6. From the data obtained, the developed method was found to be precise.

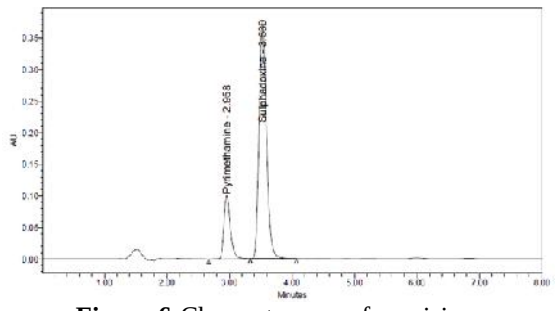

Figure 6: Chromatogram of precision

Accuracy

A study of recovery of Pyrimethamine and Sulphadoxine from spiked placebo was conducted at three different spike levels i.e.50%, 100% and 150% samples were prepared with Pyrimethamine and Sulphadoxine raw material equivalent to about the target initial concentration of Pyrimethamine and Sulphadoxine. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table 7&8. The mean recoveries of Sulphadoxine spiked were found to be in the range of 99.4% - 99.7% and Pyrimethamine from spiked were found to be in the range of 100.5-99.5%.

LOD and LOQ:

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The residual standard deviation of the regression lines and slope of the calibration curves were used to calculate the LOD and LOQ (Table no.3&4)

System suitability

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 30µg/ml. The results given in Table9 were within acceptable limits.

3. Results and Discussion

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of Pyrimethamine and Sulphadoxine in bulk dug and pharmaceutical dosage form by using the most commonly employed XBridge C-18 column with PDA-detection. The run time was set at 8min and the retention time for Pyrimethamine and Sulphadoxine was 2.896, 3.476±0.2min respectively. Each sample was injected 5 times and the retention times were same. When the concentrations of Pyrimethamine and Sulphadoxine and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r2=0.999) was observed between the concentration of Pyrimethamine and Sulphadoxine and the respective peak areas in the range 5-37.5µg /ml of Pyrimethamine and 100-750µg/ml of Sulphadoxine.

The regression equation was used to estimate the amount of Pyrimethamine and Sulphadoxine, either in tablet formulations or in validation study (precision and accuracy). For the proposed RP-HPLC method, characteristic parameters were shown in Table: 2. To analyse tablet formulations, RP-HPLC method has been developed. Pyrimethamine and Sulphadoxine tablets were analyzed as per the procedure described above. The low % RSD values (≤ 2) indicated that the method was precise and accurate. The mean recoveries found in the range of 99% – 100.5%. No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

4. Conclusion

The proposed RP-HPLC method was also validated for intra and inter-day variation. When the solution containing 25µg/ml of Pyrimethamine and 500µg/ml of Sulphadoxine was repeatedly injected on the same day, the % RSD in the peak area for six replicate injections was found to be 0.1% for Pyrimethamine and 0.8% for Sulphadoxine. Also the inter day variation (6 days and six injections) was found to be 0.01% for Pyrimethamine and 0.07% for Sulphadoxine. The results are presented in Table: 3. The % RSD values were within 2 and the method was found to be precise. It can concluded the proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Pyrimethamine and Sulphadoxine and can be reliably adopted for routine quality control analysis of Pyrimethamine and Sulphadoxine in Bulk and its pharmaceutical formulations.

Table 1: Linearity results for Pyrimethamine

Conc. (µg / ml)	5	12.5	17.5	25	30	37.5
Avg. area	226874	544892	762650	1108842	1307580	1619089
Correlation			0.999			

Table 2: Linearity results for Sulphadoxine

Conc. (µg / ml)	100	250	350	500	600	750
Avg. area	948862	2330482	3240604	4602627	5568667	6999686
Correlation			0.999			

Table 3: Precision results for Pyrimethamine

Sl no	Concentration (µg /ml)	Intraday precision (area)	Interday precision (area)
1	25	720106	1078936
2	25	719328	1078927
3	25	720111	1079968
4	25	723156	1087035
5	25	723421	1084503
6	25	725577	1082001
Mean		721950	1081895
Std Dev		2467.0	3301.8
% RSD		0.34	0.31

Table 4: Precision results for Sulphadoxine

Sl no	Concentration (µg /ml)	Intraday precision (area)	Interday precision (area)
1	500	3094406	4694010
2	500	3098555	4691388
3	500	3101099	4694073
4	500	3112125	4716215
5	500	3119704	4706324
6	500	3131409	4703315
Mean		3109550	4700888
Std Dev		14207.0	9532.7
% RSD		0.46	0.20

Table 5: Accuracy results for Pyrimethamine

Level (%)	S.No	Peak Area	Recovery (%)	Mean Recovery (%)	SD	RSD (%)
50 %	1	364238	100.80	100.53	0.5623	0.56
	2	364609	100.91			
	3	360919	99.89			
100 %	1	1132080	99.92	100.21	0.4198	0.42
	2	1132813	100.02			
	3	1137663	100.69			
150 %	1	1729008	99.54	99.51	0.0341	0.03
	2	1728321	99.48			
	3	1758903	99.53			

Table 6: Accuracy results for Sulphadoxine

Level (%)	S.No	Peak Area	Recovery (%)	**Mean** Recovery (%)	SD	RSD (%)
50 %	1	1541057	99.02	99.44	0.6781	0.68
	2	1542142	99.09			
	3	1559854	100.23			
100 %	1	4917393	100.15	100.46	0.3985	0.40
	2	4929643	100.35			
	3	4941311	100.92			
150 %	1	7463988	99.89	99.77	0.1710	0.17
	2	7462128	99.85			
	3	7619325	99.58			

Table 7: System suitability studies of Pyrimethamine and Sulphadoxine by RP-HPLC method

Property	Pyrimethamine Values	Sulphadoxine Values	Required limits
Retention time (R_t)	2.896±0.02	3.476±0.02	RSD ≤ 2%
Theoretical plates (N)	4066	4313	N > 2000
Tailing factor	1.5	1.3	T≤ 2000

Table 8: Characteristic parameters of Pyrimethamine and Sulphadoxine for the proposed RP-HPLC method

Parameters	Pyrimethamine	Sulphadoxine
Calibration range (µg/ml)	5-37.5	100-750
Detection wavelength	224nm	224nm
Mobile phase	Phosphate Buffer and Acetonitrile (70:30)	Phosphate Buffer and Acetonitrile 70:30
Retention time	2.896±0.02	3.476±0.02
Regression equation(Y*)	Y=43326x+6550	Y=9288x-701.4
Slope (m)	43326	9288
Intercept (c)	6550	701.4
Correlation coefficient (r^2)	0.999	0.999
Intraday precision (%RSD*)	0.1	0.9
Interday precision (% RSD*)	0.8	0.07
Limit of detection (mcg/ ml)	0.49	2.63
Limit of quantitaion(mcg/ml)	1.51	7.9

5. References

[1] Snyder, Kirkland and Glajch: "Practical HPLC Method Development", 2nd Edition ; Volume 24; 1997 ; Pages: 1-13,34-36,40,59-97.

[2] Synder K.L., Krikland J.J and Glajch J.L, "Practical HPLC Method Development", 2nd Edition., Wiley-Interscience Publication, USA, 1983.

[3] Beckett AH., Stenlake JB. Practical Pharmaceutical Chemistry. 4th Edition., CBS Publishers and Distributors, New Delhi, 1997.

[4] John Adamovies. Chromatographic Analysis of Pharmaceutical. 2ndEdition., Marcel Dekker Inc., New York, 2002.

[5] Astier H, Renard C, et al., Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography after automated liquid-solid extraction, Journal of Chromatography B: Biomedical Sciences and Applications. 1997; 698 (1-2), 217-223.

[6] Bergqvist Y, Eriksson M et al., Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography, Transactions of the Royal Society of Tropical Medicine and Hygiene, 1985; Volume 79, Issue-3, Pages:297-301.

[7] Meena .S and Sandhya.S.M.,Validated HPTLC Method for Simultaneous Analysis of Pyrimethamine and Sulphadoxine in Pharmaceutical Dosage Forms, Hindawi Publishing Corporation, Journal of Chemistry, Volume 2013.

[8] S. Meena, M. Sandhya., Validated spectrophotometric methods for simultaneous analysis of Pyrimethamine and Sulphadoxine in pharmaceutical dosage forms, Asian journal of

pharmaceutical and clinical research, Volume 6, Suppl 3, 2013, 121-123.

[9] Guenzi .A, Cappelletti .G., Simultaneous determination of pyrimethamine and mefloquine in human plasma by high-performance liquid chromatography with ultraviolet detection, Journal of Chromatography B: Biomedical Sciences and Applications, Volume 494, 1989, Pages 219–230

[10] Sayeed Arayne .M, najma Sultana, Farhan Ahmed Siddiqui, Simultaneous determination of pyrimethamine, sulfadoxine, mefloquine, and ibuprofen in pharmaceutical formulations by RP-HPLC, Medicinal chemistry research, 2010, Volume 19, Issue 9, pp 1043-1054.

[11] Eljaschewitsch J, Padberg J, , D Schürmann, and B Ruf, High-performance liquid chromatography determination of pyrimethamine, dapsone, monoacetyldapsone, sulfadoxine, and N-acetyl-sulfadoxine after rapid solid-phase extraction, Therapeutic Drug Monitoring, 1996, 18(5):592-597.