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Development and validation of stability-indicating method for the estimation of pyrimethamine in tablet dosage form

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ABSTRACT

A novel HPLC method was developed with precise, accurate, linear robust, rugged and validated as per ICH guidelines by using high performance liquid chromatography method used for the analysis of pyrimethamine in pharmaceutical dosage form. The HPLC method has been carried out by using Agilent zorbax SB-C8, 150 x 4.6 mm 3.5µm column. The mobile phase consist of pH 4.0 buffer: methanol in the ratio of 55:45 %v/v and the flow rate of 1.2 ml/min by the detection of UV at 280 nm. The retention time of the pyrimethamine is 4.98 min. Total chromatography runtime is 12 min. The linearity range was found to be over a concentration of 60%-160% with a correlation coefficient of 0.9999.The accuracy was found to be 99.4 to 100.7%.The projected method can be utilised for the analysis of the drug in pharmaceutical formulation.

Keywords: RP- HPLC, Pyrimethamine, Method development, Validation

INTRODUCTION

Pyrimethamine is antiparasitic drug. It is a synthetic derivative of ethyl-pyrimidine with effective antimalarial properties. Pyrimethamine is a dihydrofolate reductase inhibitor (DHFR). DHFR is a vital enzyme in the redox cycle for production of tetrahydrofolate that is required for the synthesis of DNA and proteins. This agent can be used in combination with other antimalarials for the treatment of uncomplicated falciparum malaria [1].

Pyrimethamine is an aminopyrimidine that is pyrimidine-2,4-diamine which is substituted at position 5 by a p-chlorophenyl group and at position six by an ethyl group. It is a folic acid antagonist used as an antimalarial or with a sulfonamide to treat toxoplasmosis. It has a role as an antimalarial dihydrofolate reductase inhibitor and an antiprotozoal drug. It is an aminopyrimidine and a member of monochlorobenzenes [2-3].

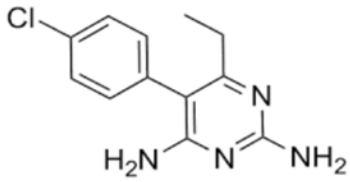

Figure 1: Structure of pyrimethamine

Literature survey shows that few HPLC [4-5], Spectrophotometric [6] and LC-MS [7-9] methods for the estimation of sulfadoxine and pyrimethamine in combination dosage form have been reported. Based on the literature survey, objective of the current study is to develop a precise, accurate, linear, robust and rugged method by using RP-HPLC, for the analysis of pyrimethamine in tablet dosage forms as per ICH guide lines Q2 R1 [10-12].

MATERIALS AND METHODS

Drugs and chemicals

Pyrimethamine Reference standard procured from USP and TLC pharmaceutical standards. HPLC grade sodium acetate trihydrate, triethylamine, glacial acetic acid and methanol and Milli-Q water obtained from Merck.

Instrumentation

A waters HPLC (E-2695 – PDA 2996) instrument equipped with Empower -3 software. Column configuration is Agilent zorbax SB-C8, 150 x 4.6 mm 3.5µm. Digilab sonicator used to dissolve and degassing. Sartorius electronic balance was used to weigh the materials. All pH adjustments were done using a Metrohm pH meter.

Mobile Phase preparation

Buffer preparation: Weighed about 6.8g of sodium acetate trihydrate and transferred into a suitable container containing 1000 ml of water. Added 2.0 ml of triethylamine sonicated to dissolve. Mixed well and adjusted the pH to 4.0 with glacial acetic acid and filtered through the 0.45 µm nylon membrane. Mixed the buffer and methanol in the ratio of 55:45 % v/v.Sonicated to degas

Diluent Preparation

1% Glacial acetic acid: Transfer 10.0 ml of glacial acetic acid into a 1000 ml container containing 500 ml of water. Diluted to volume with water and mixed well.

Diluent: Transferred 500 ml of methanol and 500 ml of 1% glacial acetic acid into a suitable container and mixed well.

Standard preparation

Weighed accurately and transferred about 25 mg of Pyrimethamine RS into a 50 ml volumetric flask. Added 30 ml of diluent sonicated to dissolve and diluted to the volume with diluent and mixed well. Further transferred 10.0 ml of above stock solution into a 50 ml volumetric flask. Diluted to the volume with diluent and mixed well

Sample preparation

Weighed 20 tablets and determined the average tablet weight. Crushed the required tablets into fine powder and mixed the powder uniformly. Weighed the powder equivalent to 100 mg of pyrimathamine into 200 ml volumetric flask. Added 140 ml of diluent and sonicated for 20 minutes with intermittent shaking. Diluted to the volume with diluent and mixed well. Centrifuged the sample solution for 10 minutes. Transferred 5.0 ml supernatant liquid into a 25 ml volumetric flask. Diluted to volume with diluent and mixed well. Filtered through 0.45µ nylon filter by discarding first 4 ml of filterate.

Chromatographic conditions

Agilent zorbax SB-C8, 150 x 4.6 mm 3.5µm column was utilized for chromatographic separation. The mobile phase composed with pH 4.0 Buffer: Methanol (55:45), at flow rate of 1.2 ml/min with run time of 12 min. Detection

wavelength at 280 nm. Retention time of Pyrimethamine was found to be 4.98 minutes. Injection volume 20 µl, Column oven thermostatically controlled with 30°C, Sampler temperature maintained at 10°C.

Method validation

The developed method was validated by following parameters precision, accuracy, linearity, specificity, robustness and as per the ICH guidelines.

System precision

To determine the system precision, standard solution was prepared as per the method. Relative standard deviations for peak area responses from five replicate injections of the standard solution, USP tailing factor and USP plate count factor for Pyrimethamine peak was reported (Table 1). The percentage relative standard deviation from five replicate standard injections should be not more than 2.0%.Acceptance criteria for USP tailing factor should be not more than 2.0 and USP plate count for Pyrimethamine peak obtained from standard solution should be not less than 2000. (Figure 1).

Method precision

To establish the precision of the assay method six individual samples of Pyrimethamine tablets were prepared. The samples were prepared as per the method (Figure 2). The assay results should be within the limit of 93.0% -107.0%.. The percentage relative standard deviation from six individual sample preparations should be not more than 3.0% (Table 2).

Linearity

Linearity of the method was performed by varying concentrations range from 60 % to 160 % of the standard concentrations were injected to HPLC system. Correlation co efficient should be not less than 0.999. (Table 3).

Accuracy

In Accuracy study the placebo was taken and varying amounts of Pyrimethamine drug substance representing 50 % to 200 % of standard concentration of Pyrimethamine were added to the flasks. The spiked samples were prepared as per the procedure. The individual % recovery and average % recovery at each level must be between 97.5%-102.5% for Pyrimethamine. The overall average percentage recovery should be between 98.0%-102.0% and the overall % RSD should be not more than 3.0% (Table 4).

Specificity

A forced degradation study is accomplished in order to demonstrate that the method is stability indicating. Pyrimathamine finished product was stressed under Acid, base, peroxide, Uv light and heat. Blank, standard, control and stress sample solutions were prepared and injected into the chromatographic system for identification and impurity interference with the Pyrimethamine peak. The acceptance criteria for specificity study, any secondary peak arising from forced degradation study should not interfere with Pyrimethamine peak, no interference should be observed from diluent, placebo and all the known impurities at the retention time of Pyrimethamine peak, peak purity analysis using PDA detector should demonstrate peak homogeneity. Purity threshold for stress samples should be higher than the purity angle (Table 5).

Robustness

In robustness study, standard solution was prepared and injected into the chromatographic system as per the conditions specified in the method. The same standard solution was re injected by changing method parameters. A set of system suitability data was calculated for standards injected under altered method conditions and compared against the values generated under normal conditions (Table 6).

Ruggedness

The ruggedness of the assay method was determined by injecting six individual sample solutions of Pyrimethamine tablets prepared by a second analyst using different HPLC system and a different column on a different day. The assay results should be within the limit of 93.0% - 107.0%. The % RSD from six individual sample preparations should be not more than 3.0% and difference in mean % assay between intermediate precision and method precision should be not more the 3.0% (Table 7).

RESULTS AND DISCUSSION

Table 1: System precision data of Pyrimethamine

S.no	Name	Retention time	Area	USP Tailing	USP Plate count
1	Standard-1	4.983	2655768	1.5	7291
2	Standard-2	4.984	2660602	1.5	7299
3	Standard-3	4.985	2657621	1.5	7319
4	Standard-4	4.977	2656435	1.5	7312
5	Standard-5	4.980	2667902	1.5	7263
Mean			2659666		
% RSD			0.2		

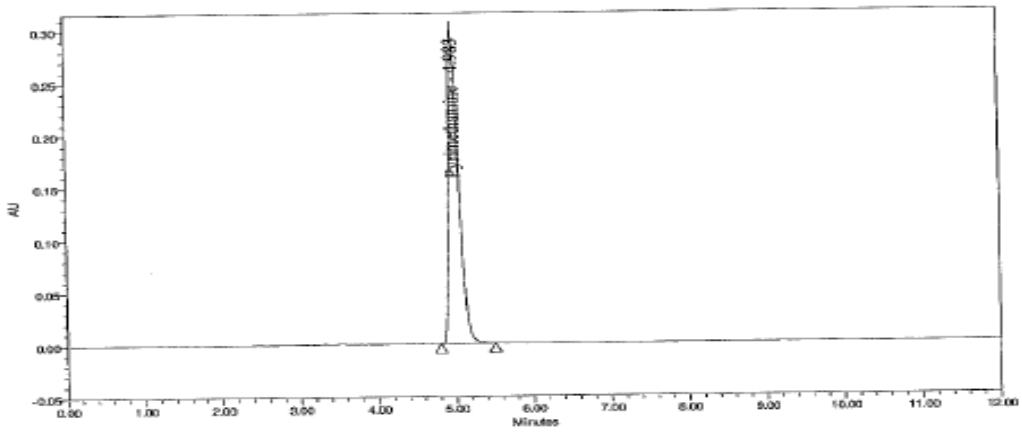

Figure 1: Typical chromatogram of standard

Table 2: Method precision data of Pyrimethamine

S.no	Name	Area	Assay
1	Sample-1	2694083	101.6
2	Sample-2	2688431	101.4
3	Sample-3	2697234	101.7
4	Sample-4	2669466	100.6
5	Sample-5	2680835	101.0
6	Sample-6	2673943	100.8
Mean			101.2
% RSD			0.4

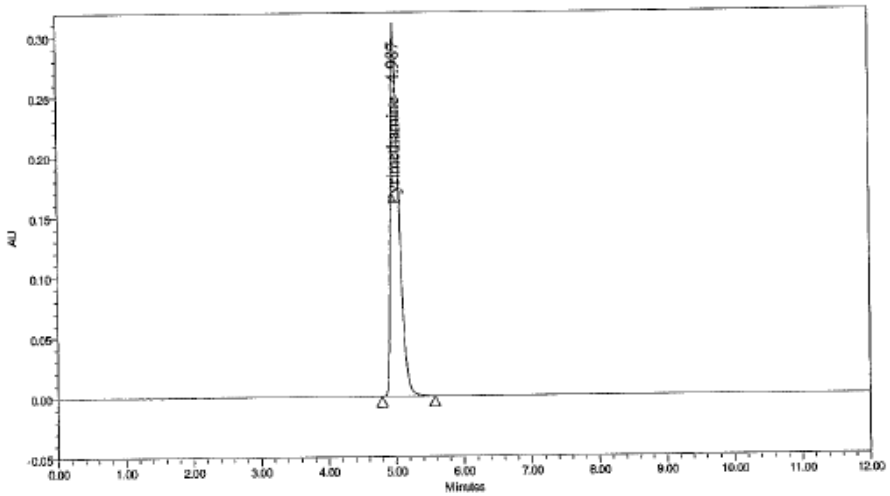

Figure 2: Typical chromatogram of sample

Table 3: Linearity data for Pyrimethamine

S.no	Name	Area
1	Linearity - 60%	1673673
2	Linearity - 80%	2249516
3	Linearity - 100%	2812502
4	Linearity - 120%	3364462
5	Linearity - 160%	4469568
Correlation coefficient square = 0.9999		

Table 4: Accuracy data for Pyrimethamine

S.no	Name	Area	Amount added µg/ml	Amount found µg/ml	% Recovery	Mean	% RSD
1	Recovery 50% -1	1352296	50.3600	50.2736	99.8	99.7	0.2
2	Recovery 50% -2	1359520	50.6700	50.5421	99.7		
3	Recovery 50% -3	1340653	50.1300	49.8407	99.4		
1	Recovery 100% -1	2712905	100.4300	100.8562	100.4	100.4	0.1
2	Recovery 100% -2	2714725	100.5300	100.9239	100.4		
3	Recovery 100% -3	2708215	100.4200	100.6818	100.3		
1	Recovery 200% -1	5419093	200.7800	201.4627	100.3	100.5	0.2
2	Recovery 200% -2	5419540	200.2400	201.4793	100.6		
3	Recovery 200% -3	5437060	200.7700	202.1306	100.5		
Overall average recovery %						100.2	

Table 5: Specificity data for Pyrimethamine

S.no	Name	Purity angle	Purity threshold
1	Control sample	0.118	0.264
2	Acid degradation sample 5N HCl/80°C/5 hrs	0.648	1.114
3	Base degradation sample 1N NaOH/80°C/4 hrs	0.097	0.266
4	Peroxide degradation sample 3% H2O2/80C/4 hrs	0.105	0.263
5	Heat degradation /80°C/16 hrs	0.126	0.266
6	Uv light degradation sample Uv light/16 hrs	0.139	0.267
7	Spiked sample	0.147	0.267

Table 6: Robustness data for Pyrimethamine

Parameter	% RSD
Column temperature plus	0.05
Column temperature minus	0.02
Flow rate plus	0.20
Flow rate minus	0.10
Mobile phase composition plus	0.10
Mobile phase composition minus	0.04

Table 7: Ruggedness data for Pyrimethamine

S.no	Name	Area	Assay
1	Sample-1	2739837	102.1
2	Sample-2	2688322	101.3
3	Sample-3	2721484	101.4
4	Sample-4	2714373	101.1
5	Sample-5	2727716	101.1
6	Sample-6	2715121	100.5
Mean			101.2
% RSD			0.5
The % Assay between method precision and ruggedness = 0.0 %			

CONCLUSION

The proposed RP-HPLC method was found to be precise, linear, accurate, specific, robust and rugged for the estimation of Pyrimethamine in tablets dosage form. The developed method is suitable for regulatory approach and complies with regulatory requirements as per ICH guidelines. Hence this method easily adopted for routine analysis of Pyrimethamine.

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