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Compendial invitro - Method validation for pyrimethamine tablet dosage form by UV spectrophotometric method

Kannan Jakkan*[1], Nataraj palaniyappan[2], Ravisankar Mathesan[3]

[1]*Department of Chemistry, Jaipur National University, Rajashthan. India*
[2]*Novitium Pharma LLC, New jersey, USA.*
[3]*Vinayaka missions Research foundation, Salem, Tamilnadu, India.*

***Corresponding Author: Kannan jakkan**
Email: kannanparform@gmail.com

ABSTRACT

Dissolution testing has developed in the pharmaceutical sector as a crucial key to illustrate drug released from the dosage forms. Pyrimethamine tablets USP, 25mg prescribed for anti-parasitic and to treat the toxoplasmosis. It has dissolution method in official monograph. The aim of current study was to verify the compendial dissolution method to adapt as In-house method. Outcomes from the dissolution method verification show that the method is suitable for in house laboratory. In vitro dissolution tests were performed using official method conditions. Filter compatibility study was evaluated. The test conditions were 0.1N HCl (900 mL at 37 ± 0.5 °C) as dissolution medium, paddle method (USP-II), 50 rpm, and 45 minutes. Results were satisfactory. The UV spectroscopy method was used to record the absorbance. The method shows linearity (r2 = 0.99986) in the concentration range of 50% to 150% of standard concentration. The % recoveries were good, ranging from 99.70% to 102.50%. The validated dissolution test is acceptable for its purpose and it can be applied in in-house analysis

Keywords: Pyrimethamine, Dissolution, Method validation, Method verification, UV spectrophotometry

INTRODUCTION

Pyrimethamine is one of the drug used as folic acid antagonists and it contains potent anti-malarial property. Pyrimethamine restricts with the regeneration of tetrahydrofolic acid from dihydrofolate by inhibiting the enzyme dihydrofolate reductase. Tetrahydrofolic acid is key factor for DNA and RNA synthesis in many species, which includes including protozoa[1]. IUPAC name of pyrimethamine is 5,4-chlorophenyl-6-ethyl-2,4-pyrimidinediamine. The combination of pyrimethamine along with sulfonamide and sulfadoxine, which interferes the dihydrofolate synthesis by inhibit the fusion of PABA into the dihydrofolate. It leads produce consecutive blockage of tetrahydrofolate synthesis[2]. A plasmodium enzyme induces folic acid synthesis contrast from enzymes found in other organisms. The bifunctional protein which is present in plasmodium sp. Induces the phosphorylation of 6-hydroxymethyl-7,8-hydropterin and the integration of PABA into dihydropteroic acid. A second bifunctional enzyme induces the reduction of dihydropteroic acid and thymidylic acid synthesis. The drug combination seems to have enhanced drug-mediated interruption of folic acid in Plasmodium sp[3, 4].(Figure 1)

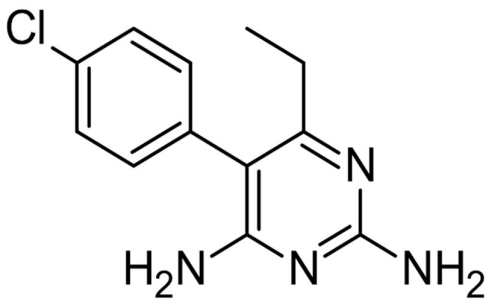

Fig 1: Structure of pyrimethamine

A literature studies show that several methods were reported by different techniques such as HPLC [5-7], UV[8] and LC-MS[9-11] for the analysis of pyrimethamine along with its different combinations. The verification process for compendial test procedures is the assessment of whether the procedure can be used for its intended purpose.[10] Based on the literature study there is no evidence for the validation of pyrimethamine tablets USP, 25 mg compendial dissolution method. Hence the objective of the current study is to perform compendial method validation for pyrimethamine tablets USP, 25 mg by UV according to ICH guidelines and USP chapter 1226[12-15].

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

Dissolution - Distek dissolution model 2100c and Schimadzu UV-1800 model was utilized for the study.

Preparation of dissolution medium

Added 51 ml of Hydrochloric acid into a 6000 ml of water. Mixed well and sonicated to degas.

Standard preparation

Weighed accurately about 28 mg of Pyrimethamine RS into a 100 ml volumetric flask. Added 10 ml of methanol sonicated to dissolve and diluted to the volume with dissolution medium. Further pipetted out 5.0 ml of standard stock and transferred into 50 ml volumetric flask. Diluted to the volume with dissolution medium and mixed well.

Sample preparation

Randomly selected and weighed six tablets individually. Recorded the weight of each tablet. Placed the tablets into individual dissolution vessels. Run the dissolution by applying the parameters mentioned in Table.1

Carefully withdrawn 10 ml of sample and filtered through 0.45 µm nylon filter by discarding first few ml of the filtrate. UV absorbance recorded for samples by using the parameters mentioned in Table.2

Method validation

Compendial method verified by following parameters precision, linearity and range, accuracy, filter study, solution stability and specificity as per the ICH guidelines.

System precision

System precision determined by standard solution was prepared as per the method. Relative standard deviations for absorbance from six measurements of the standard solution was calculated and reported in Table.3.The % RSD for absorbance from six replicate measurements of Pyrimethamine standard solution not more than 3.0%.

Linearity and range

Linearity of the method was executed by solutions of pyrimethamine at varying concentrations ranging from 50 % to 150 % of the standard concentration of 28µg/ml of pyrimethamine were measured for the UV absorbance. The correlation coefficient square must be not less than 0.997 (Table 4).

Method precision

Precision of the dissolution method was determined by measuring the absorbance of six samples of pyrimethamine tablets .The samples were prepared as per the method. The results should be within the specification limits. The %RSD for %dissolved from six units should be not more than 10%. (Table.5)

Solution stability

The standard and sample solutions were stored at room temperature and the absorbance measured at initial and 22 hours of time intervals. The % assay of standard at time point should be between 97.0% to 103.0%.The absolute difference in % dissolved between the initial and the time point for

sample should be not more than 3.0 %. (Table.6)

Specificity

To verify the specificity of the dissolution method, blank (dissolution medium) and placebo solutions were prepared and measured the absorbance at same wavelength in the UV system. The absorbance of blank should be not more than ±0.002 and the interference from placebo should be not more than 2.0 %. (Table.7)

Method Accuracy

The placebo was taken and varying amounts of Pyrimethamine representing 50 %, 100% and 125 % of sample concentration of Pyrimethamine (28µg/ml) were included into the flasks. The spiked samples were prepared as per the procedure in triplicate and measured the absorbance. The individual and average recovery at each level must be between 90%-110% .Overall average recovery should be between 95.0%-105.0% (Table 8).

Filter study

The sample solution of pyrimethamine tablets sample was filtered by discarding 2ml,4ml,6ml and 8ml of the filtrate by using 0.45µm nylon filter and the samples were injected. Unfiltered centrifuged solution was also prepared and injected. Compared the results of filtered samples with that of centrifuged sample results. The difference in %dissolved should be not more than 3.0 (Table 9).

RESULTS AND DISCUSSION

Table 1: Dissolution parameters

Mode	UV
Dissolution medium	0.1N HCl
Cell path length	10 mm
Wave length	273 nm
System suitability	The % RSD for absorbance from six replicate measurements of pyrimethamine standard solution should not be not more than 3.0%.

Table 2: UV Instrument parameters

Apparatus	USP Apparatus II (Paddle)
RPM	50
Dissolution medium	0.1N HCl
Volume	900 ml, at 37°c ± 0.5°c
Time	45 minutes
Sample collection volume	10 ml

Table 3: System precision results

Reading no	Absorbance
1	0.8827
2	0.8781
3	0.8786
4	0.8817
5	0.8787
6	0.8790
Mean	0.8798
% RSD	0.22

Table 4: Linearity data for Pyrimethamine

Sample No	Name	Absorbance
1	Linearity - 50%	0.4495
2	Linearity - 80%	0.7066
3	Linearity - 100%	0.8825
4	Linearity - 120%	1.0643

5	Linearity - 150%	1.3139
Correlation coefficient square = 0.99986		

Table 5: Method precision data of Pyrimethamine

Sample No	Absorbance	% Dissolved
Method precision-1	0.8685	99
Method precision-2	0.9035	103
Method precision-3	0.8902	102
Method precision-4	0.8887	102
Method precision-5	0.8977	103
Method precision-6	0.8745	100
Mean		101
% RSD		1.51

Table 6: Solution stability data of Pyrimethamine

Standard solution stability at room temperature		
Time	**Absorbance**	**% Dissolved**
22 hours	0.8737	99.7
Sample solution stability at room temperature		
Time	**% Dissolved**	**Absolute difference in % Dissolved**
Initial	99	NA
22 hours	100	1

Table 7: Specificity data of Pyrimethamine

Sample name	Absorbance
Blank	0.0002
Placebo	0.1%

Table 8: Accuracy data for Pyrimethamine

S.no	Name	Absorbance	Amount added µg/ml	Amount found µg/ml	% Recovery	Mean
1	Accuracy 50% -1	0.4468	13.9	14.2	102.5	102
2	Accuracy 50% -2	0.4421	13.9	14.1	101.4	
3	Accuracy 50% -3	0.4447	13.9	14.2	102.0	
1	Accuracy 100% -1	0.8697	27.8	27.7	99.7	101
2	Accuracy 100% -2	0.8802	27.8	28.0	100.9	
3	Accuracy 100% -3	0.8825	27.8	28.1	101.2	
1	Accuracy 125% -1	1.0965	34.7	34.9	100.6	101
2	Accuracy 125% -2	1.0979	34.7	35.0	100.7	
3	Accuracy 125% -3	1.0977	34.7	35.0	100.7	
Overall average recovery %						101

Table 9: Filter study data for Pyrimethamine

S.no	Sample Name (0.45µm nylon filter)	% Dissolved	% Difference
1	Centrifuged	96	NA
2	2 ml discarded	96	0
3	4 ml discarded	96	0
4	6 ml discarded	97	1
5	8 ml discarded	96	0

CONCLUSION

Results from method precision, linearity, accuracy, specificity, filter study and solution stability shows the compendial dissolution method for pyrimethamine tablets USP, 25 mg can be adoptable to in-house analysis. Hence there is no need to develop new dissolution method for Pyrimethamine tablets USP, 25 mg dissolution.

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