RESEARCH ARTICLE ISSN: 2349-2678

Contents lists available at www.ijpba.in

*International Journal of Pharmaceutical and Biological Science Archive*

Index Copernicus Value: 65.90

Volume 6 Issue 2; March-April; 2018; Page No. 06-11

# METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF PYRIMETHAMINE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM BY USING UV SPECTROSCOPY

**A. Sandhya*, B. Siva sai kiran, M. Suneetha, Sk.Muneer, M. Mahesh**

Department of Pharmaceutical analysis,

JNTUA - Oil Technological and Pharmaceutical Research Institute,

Ananthapuramu - 515001, A.P, India.

**ABSTRACT**

A rapid and novel spectrophotometric method has developed and validated for the estimation of pyrimethamine in bulk and its tablet dosage form. The absorption maximum was found to be 287nm. This method obeys Beer's law the linearity concentration range from 2-12µg/ml with correlation coefficient was found to be 0.999. The accuracy and precision of the method was determined and validated according to ICH guidelines. The method shows good reproducibility with percent relative standard deviation (% RSD) ≤ 2. The validated parameters were found to be within the limits, the proposed method was recommended for routine quality control analysis for the estimation of pyrimethamine in bulk and its tablet dosage form.

**KEY WORDS:** Pyrimethamine, Validation, beer's law, ICH guidelines.

## INTRODUCTION:

Pyrimethamine inhibits the dihydrofolate reductase of plasmodia [1-5] and there by blocks the biosynthesis of purines and pyrimidines [6-8], which are essential for DNA synthesis and cell multiplication. [9-12].

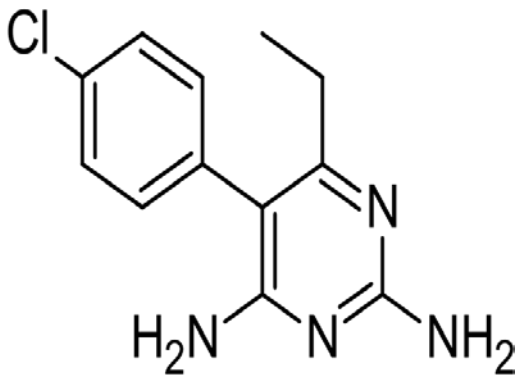


**Fig 1: Chemical structure of Pyrimethamine**

## MATERIALS AND METHODS:

### Chemicals and Reagents:

The sample Pyrimethamine (Daraprim 25mg) was procured from Merc Laboratories limited, Mumbai, India. Solvent (methanol) used was of analytical grade.

### Instrument specifications:

The sample analysis was performed by using UV visible double beam spectrophometer of Shimadzu 1800 make having deuterium lamp associated with spectra treats and enabled with UV probe software.

## METHODOLOGY:

### Preparation of stock solutions and sample solutions:

### Preparation of stock solution:

Pyrimethamine pure drug 10 mg was weighed and transferred to a 10 ml volumetric flask and dissolved It was dissolved properly and diluted up to the mark with diluent (50 % Methanol) to obtain final concentration of 1000 µg/ml. 10µg/ml solution was prepared from the stock solution was prepared using 50% methanol.

*Corresponding author: A. Sandhya |

**Preparation of standard solution:**

Pyrimethamine pure 100 mg was weighed and transferred to a 100 ml volumetric flask and dissolved in methanol. It was dissolved properly and diluted up to the mark with diluent to obtain final concentration of 1000 µg/ml. 100µg/ml solution was prepared from the stock solution was prepared using Methanol which was used as working standard.

**Preparation of working solutions:**

From the standard stock solution of Pyrimethamine appropriate aliquots were pipetted out in to 10 ml volumetric flasks and dilutions were made with distilled water to obtain working standard solutions of concentrations from 2-12µg/ml. Absorbance for these solutions were measured at 287 nm. The standard solution analytical concentration range was found to be 2-12 µg/ml

**RESULTS AND DISCUSSION**:

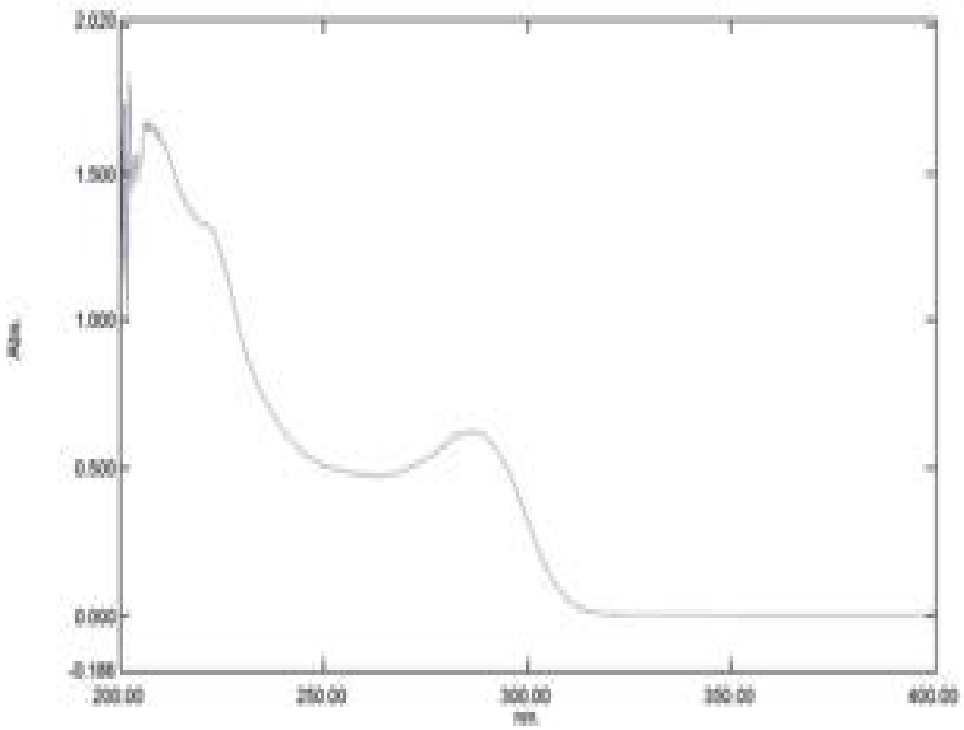


**Fig 2: UV spectra of Pyrimethamine**

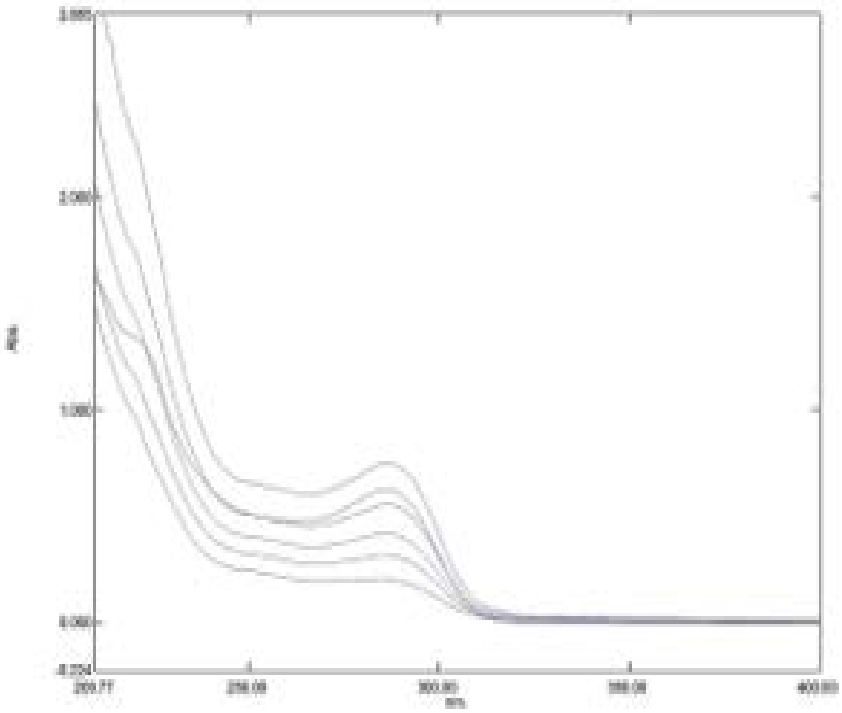

**Fig 3: Overlay UV spectra of Pyrimethamine**

**Table No 1: Results of calibration curve at 287nm for Pyrimethamine**

| S. No | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1 | 2 | 0.206 |
| 2 | 4 | 0.316 |
| 3 | 6 | 0.421 |
| 4 | 8 | 0.554 |
| 5 | 10 | 0.643 |
| 6 | 12 | 0.549 |

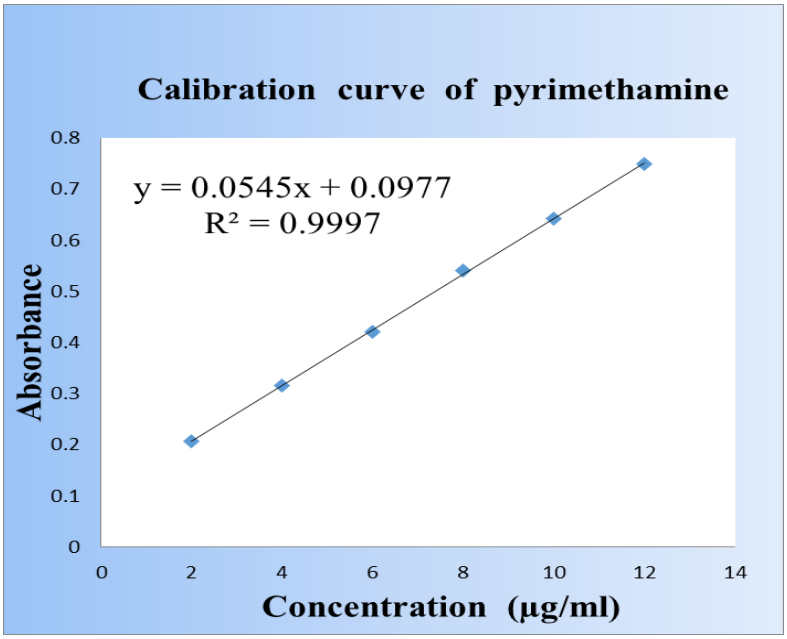


**Fig 4: Linearity curve for Pyrimethamine**

**Table No 2: Precision results of pyrimethamine**

| S. No. | Precision | |
|---|---|---|
| | Intra day | Inter day |
| 1. | 0.544 | 0.543 |
| 2. | 0.543 | 0.544 |
| 3. | 0.541 | 0.546 |
| 4. | 0.542 | 0.541 |
| 5. | 0.545 | 0.542 |
| 6. | 0.542 | 0.544 |
| **Mean** | 0.5421 | 0.5433 |
| **Std dev** | 0.0015 | 0.0019 |
| **% RSD** | 0.2900 | 0.3629 |

**Table No 3: Determination of accuracy results for pyrimethamine**

| Sr. No | Spike Level | Absorbance | µg/ml Added | µg/ml Found | % Recovery |
|---|---|---|---|---|---|
| 1 | 50 % | 0.32 | 2.28 | 2.37 | 100.718 |
| 2 | 100 % | 0.54 | 8.02 | 8.01 | 99.879 |
| 3 | 150 % | 0.74 | 16.52 | 16.64 | 100.243 |

The mean % recovery was found to be 99.58 %

**Table no.4: Results for detection and quantification limits**

| Sr. No. | Parameter | Standard deviation | Slope | LOD & LOQ (µg/ml) |
|---|---|---|---|---|
| 1 | Limit of detection | 0.0015 | 0.0545 | 0.090 |
| 2 | Limit of Quantification | | | 0.275 |

**Table No. 5: Results of Robustness studies**

| Sr. No | Robust parameter | Wavelength (nm) | Absorbance ± Std Deviation (n=3) | % RSD |
|---|---|---|---|---|
| 1 | Wavelength (±2nm) | 285 | 0.538±0.00163 | 0.3035 |
| 2 | | 287* | 0.54±0.00125 | 0.2304 |
| 3 | | 289 | 0.546±0.0017 | 0.3099 |

**Table No 6: Summary of validation parameters obtained for proposed UV**

| Sr. No. | Parameters | Results |
|---|---|---|
| | Absorption Maxima (nm) | 287 |
| | Beer's-Lambert's range (µg/ml) | 2-12(µg/ml) |
| | Regression equation (y)* | y = 0.0545x + 0.0977 |
| | Slope (b) | 0.0545x |
| | Intercept (a) | 0.0977 |
| | Correlation coefficient (r2) | 0.999 |
| | Intraday precision (% RSD)** | 0.290 |
| | Interday precision (% RSD)** | 0.362 |
| | Accuracy (% mean recovery) | 100.28 |
| | Limit of detection (µg / ml) | 0.090(µg / ml) |
| | Limit of quantification (µg / ml) | 0.275(µg / ml) |
| | Assay of tablets (%Purity) | 99.44% |

*Y = bx + a where x is the concentration of Pyrimethamine in (µg / ml) and Y is the absorbance of the respective λ max.

**Average of Six determinations

## CONCLUSION:

A novel, precise, economical, accessible, reliable and reproducible method for estimation of Pyrimethamine in bulk and its tablet dosage form using UV method was developed and was validated as per ICH guidelines. The RSD values for all the validation parameters were found to be less than two, indicating that the proposed UV method were trustworthy. This method has ample scope and application in industry for estimation of Pyrimethamine.

## AKNOWLEDGEMENT:

I thank full to Neon Laboratories limited, Mumbai, India for providing the bulk drug.

**REFERENCES:**


**1** Van Ruyven, C.B. Rothova, A prospective, randomized trial of pyrimethamine and azithromycin vs pyrimethamine and sulfadiazine for the treatment of ocular toxoplasmosis. *Am. J;* 2002; 134: 34

**2** Anderson, A.C, Targeting DHFR in parasitic protozoa. *Drug Discov. Today;* 2005; 10:121.

**3** N. Kalpesh Patel, Development and validation of RP-HPLC method for simultaneous determination of Pyrimethamine and sulphadoxine in bulk and pharmaceutical formulations. Pharmacist (New Delhi, India); 2011; 10(3):37-40.

**4** Weidekamm.E,Determination of Pyrimethamine in human plasma after administration of Fansidar or Fansidar-mefloquine by means of high-performance liquid chromatography with fluorescence detection. Journal of Chromatography, Biomedical Applications*;* 1982; 230(1);107-14.

**5** Guiping Yu, Determination of Pyrimethamine in fish filets by HPLC Qian. 2005; 15(6); 697-698.

**6** L. I. armats evtichnii Zhurnal (Kiev), Spectro-photometric determination of Pyrimethamine Rapaport. 1968; 23(3);60-1.

**7** The Indian Pharmacopoeia, Vol. III, the Indian Pharmacopoeia Commission, 5th ed., India: NISCAIR Press, 2007: 1011, 1012, 1142.

**8** USP 25-NF 20, Validation of Compendial Methods Section (1225).United States Pharmacopoeial Convention, Rockville, Maryland, USA; 2002:2256.

**9** O'Neil MJ. The Merck Index: an Encyclopedia of Chemicals, Drugs and Biologicals. 14th Ed. Whitehouse Station: Merck Research Laboratories, Division of Merck And Co., Inc.; 2006: 1125.

**10** P. Saritha, T. Charan Singh and C. Gyana Kumari, spectrophotometric determination of pyrimethamine in pure and pharmaceutical forms.world journal of pharmacy and pharmaceutical sciences; 2015; 4 (9) ;,pg no 1077-1088 .

**11** Raghunath D. Wadulkar,validated stability indicating spectrophotometric method foe estimation of degradarion behaviour of pyrimethamine and sulfadoxim in tablet formulation .Asian journal of pharmaceutical technology and innovation; 2016 ;vol 4 (20) ; pgno 16-25.

**12** Hagga M. A. Mohamed, Mohd. Imran1, Monadil H. M. Ali1, Mohammed F. Abdelwahab and Abdullrahman A. Alhaj,A UV-Spectrophotmetric Chemometric Method for the Simultaneous Determination of Sulfadoxine and Pyrimethamine in Tablets .Asian Journal of Pharmaceutical Research and Health Care ; year2016;vol 8 issue(3) ,pg no :76-83.

**13** M. sandhya. meena ,validated spectrophoto-metric methods for simultaneous analysis of pyrimethamine and sulphadoxine in pharmaceutical dosage forms,. Asian Journal of Pharmaceutical Clinical Research Vol 6, year 2013, Pg no: 121-123.