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Research Article

# VALIDATED SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ANALYSIS OF PYRIMETHAMINE AND SULPHADOXINE IN PHARMACEUTICAL DOSAGE FORMS

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**ABSTRACT**

Objective: Two simple, accurate and reproducible spectrophotometric methods have been developed and validated for simultaneous estimation of pyrimethamine and sulphadoxine in combined dosage form. Methods: Pyrimethamine shows maximum absorbance at 285.6 nm and sulphadoxine shows maximum absorbance at 273.4 nm. In dual wavelength method, two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug. Pyrimethamine shows equal absorbance at 278 and 295 nm, where the difference in absorbance was measured for determination of sulphadoxine. Similarly, difference in absorbance at 246 and 298 nm were measured for determination of pyrimethamine. For multicomponent mode method; the sampling wavelengths selected were 285.6 and 273.4 nm. Results: Linearity for detector response was observed in the concentration range of 3-18 µg/mL for pyrimethamine and 7-42 µg/mL for sulphadoxine for method I, 5-30 µg/mL for pyrimethamine and 10-60 µg/mL for sulphadoxine for method II respectively. Accuracy and precision studies were carried out and results were satisfactory. The proposed methods were validated as per ICH guidelines. Conclusion: The developed methods are simple, precise, rugged and economical. The utility of the methods has been demonstrated by analysis of commercially available formulations.

**Keywords:** Pyrimethamine, Sulphadoxine, Dual wavelength method, Multicomponent analysis, Method validation.

## INTRODUCTION

Pyrimethamine (PYR; 5-(4-cholorophenyl)-6-ethyl pyrimidine 2, 4-diamine; sulphadoxine (SUL; 4-amino-N-[5, 6-dimethoxy-4-pyrimidinyl] benzene sulphonamide; (Figure. 1) are used as antimalarial drugs [1, 2]. Synergism between PYR and SUL is explained by inhibition of two steps in an essential metabolic pathway. The two steps involved are the utilization of p-amino benzoic acid for the synthesis of dihydropteroic acid, which is catalysed by dihydropteroate synthase and inhibited by SUL and the reduction of dehydrofolate to tetrahydrofolate, which is catalyzed by dihydrofolate reductase and inhibited by PYR [3].

Individual PYR and SUL as well as combination of both are officially recognized in the Pharmacopoeia. The Pharmacopoeia describes a titrimetric method for determination of PYR and SUL in their individual tablet formulation and high performance liquid chromatography (HPLC) for tablet formulation. The other reported methods for quantification of PYR and SUL individually or in combination with other drugs from dosage form or in biological fluids include various analytical methods such as spectrophotometry [4, 5], HPLC with UV [6-12], HPLC with MS [13], supercritical fluid chromatography [14] and spectrofluorimetry [15].

However, there is no work reported concerning simultaneous spectrophotometric determination of PYR and SUL by proposed methods. The aim of present investigation is to develop simple and economical spectrophotometric methods with greater precision, accuracy and sensitivity for simultaneous estimation of PYR and SUL in pure and tablet dosage forms and validate in accordance with ICH guidelines [16].

## EXPERIMENTAL

### Chemicals and Reagents

Pharmaceutical grade PYR and SUL were obtained as generous gift from Medopharm Pharmaceuticals, Karnataka, India. Chemicals were of AR grade from Merck Chemicals, India. Combined tablet dosage forms were procured from the local market (labeled to contain PYR 25 mg and SUL 500 mg per tablet). Spectroscopy grade methanol was used throughout study.

### Equipment

A double beam UV-visible spectrophotometer (Shimadzu, Japan) model UV-170 with quartz cell 1 cm path length, connected to HP computer version 2.21 was used. Shimadzu balance (AUW-120D) was used for all weighing.

### Standard stock solutions

Stock solutions (1 mg/mL) were prepared for PYR and SUL separately in methanol. From these stock solutions, sub stock solutions (100 µg/mL) were prepared for both drugs. From these sub stocks, eight mixed standards were prepared having PYR and SUL in the ratio of 1:20 (as in combination tablet).

### Sample preparation

Twenty tablets were accurately weighed and powdered. Quantities of tablet powder equivalent to label claim of PYR and SPDX were accurately weighed and transferred to 50 mL volumetric flask, dissolved in 25 mL methanol and vortexed for 15 minutes. The volume made up to 50 mL with methanol and mixed well. Solution obtained was filtered through Whatmann filter paper no. 42, diluted with same solvent to get the concentration within linearity range and used for the measurement of absorbance.

### Method I

### Dual wavelength method

The spectrum of PYR shows identical absorbance at 278 nm ($\lambda_1$) and 295 nm ($\lambda_2$) while that of SUL reveals same absorbance at 246 nm ($\lambda_3$) and 298 nm ($\lambda_4$), therefore wavelengths at $\lambda_1$, $\lambda_2$ and $\lambda_3$, $\lambda_4$ were selected for analysis of PYR and SUL respectively (Fig. 1). The concentrations of two drugs were calculated each from corresponding regression equation.

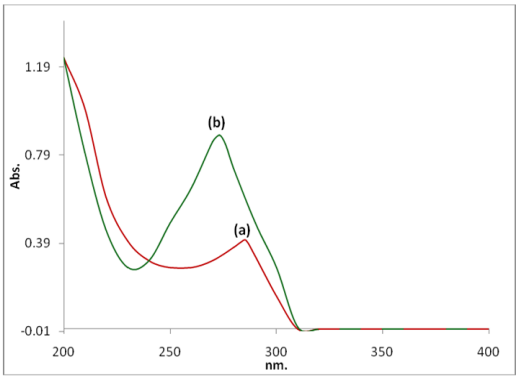


**Figure 1: Overlain spectra of (a) PYR and( b) SUL**

**Method II**

**Multicomponent mode method**

Five mixed standard solutions of each containing PYR and SPDX in the concentration ratio of 1:20, 1.5:30, 2:40, 2.5:50 and 3:60 as in commercial tablet was prepared in methanol and scanned over the range of 200-400 nm in the multicomponent mode, which were further processed for analysis (Fig. 2). The overlain spectrum obtained was employed to determine the concentration of drugs in sample solutions with reference to mixture standards.

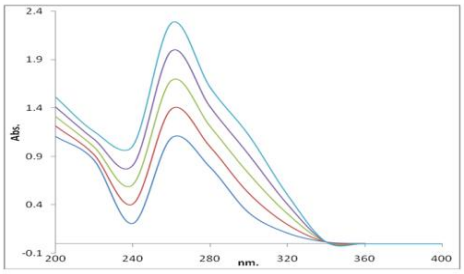


**Figure 2: Spectra of PYR and SUL in multicomponent mode**

**RESULTS AND DISCUSSION**

The main aim of this work was to establish and validate simple, sensitive and accurate spectrophotometric methods as substitutes for chromatographic and other methods reported for the simultaneous determination of PYR and SUL in bulk and dosage form with satisfactory precision.

**Linearity and sensitivity**

The linearity of the methods were evaluated by analysing eight concentrations (3-18 µg/mL PYR and 7-42 µg/mL SUL for method I; 5-30 µg/mL PYR and 10-60 µg/mL SUL for method II respectively) of each drug in triplicate. Table 1 reveals the correlation coefficients along with standard deviation of slope ($S_b$) and intercept ($S_a$). The limit of detection (LOD) and quantification (LOQ) were calculated using standard deviation of response and slope of calibration curve.

**Table 1: Optical characteristics for PYR and SUL by proposed methods**

| Parameters | Multicomponent mode | | Dual wavelength | |
|---|---|---|---|---|
| | PYR | SUL | PYR | SUL |
| Range of linearity (µg/mL) | 5-30 | 10-60 | 3-18 | 7-42 |
| $S_a$ | 0.0017 | 0.00044 | 0.00081 | 0.00018 |
| $S_b$ | 0.00033 | 0.00013 | 0.0054 | 0.00026 |
| Correlation coefficient (r) | 0.9987 | 0.9994 | 0.9988 | 0.9997 |
| LOD (µg/mL) | 0.18 | 2.73 | 0.49 | 0.93 |
| LOQ (µg/mL) | 0.87 | 4.86 | 1.13 | 1.99 |
| Regression coefficient ($r^2$) | 0.978 | 0.989 | 0.999 | 0.988 |

**$S_a$-Standard deviation of intercept of regression line, $S_b$ = Standard deviation of slope of regression line**

**Precision**

For method I, Intraday precision (repeatability) was calculated using three concentrations of PYR (3, 9 and 18 µg/mL) and SUL (7, 21 and 42 µg/mL) and for method II also three concentrations of PYR (5, 15 and 30 µg/mL) and SUL (10, 30 and 60 µg/mL) were used and analyzed in triplicate. The inter day precision (reproducibility) was repeated three times on three different days for analysis of three different concentrations for both drugs. % RSD (Table 2) for PYR and SUL for both methods ranged from 0.63 to 1.97 indicating repeatability and reproducibility.

**Table 2: Intraday and inter day precision study**

| Method | Precision | Amount taken (µg/mL) | | % RSD | |
|---|---|---|---|---|---|
| | | PYR | SUL | PYR | SUL |
| Multicomponent | Intraday | 5 | 10 | 1.97 | 0.76 |
| | | 15 | 30 | 0.83 | 0.81 |
| | | 30 | 60 | 0.73 | 0.63 |
| | Inter day | 5 | 10 | 1.25 | 0.89 |
| | | 15 | 30 | 1.11 | 0.88 |
| | | 30 | 60 | 0.96 | 1.36 |
| Dual wavelength | Intraday | 3 | 7 | 1.65 | 0.97 |
| | | 9 | 21 | 1.88 | 0.84 |
| | | 18 | 42 | 0.96 | 1.47 |
| | Inter day | 3 | 7 | 1.22 | 0.96 |
| | | 9 | 21 | 1.35 | 1.22 |
| | | 18 | 42 | 1.08 | 0.69 |

***Mean of three determinations**

**Accuracy**

Accuracy of the methods were assured by standard addition technique, which was performed by addition of known amounts of pure PYR and SUL to known concentrations of tablet powder, and

analyzed by proposed methods in triplicate. Table 3 indicates good accuracy and shows no interference from tablet excipients.

**Table 3: Recovery study of proposed methods**

| Drug | Amount taken (µg/mL) | Amount added (µg/mL) | Amount recovered ± SD* (µg/mL) | % RSD | Amount recovered ± SD* (µg/mL) | % RSD |
|---|---|---|---|---|---|---|
| | | | Multicomponent mode | | Dual wavelength | |
| PYR | 10 | 08 | 17.85 ± 0.108 | 0.61 | 17.89 ± 0.096 | 0.54 |
| | | 10 | 19.85 ± 0.097 | 0.49 | 20.06 ± 0.085 | 0.42 |
| | | 12 | 22.03 ± 0.099 | 0.45 | 21.89 ± 0.063 | 0.29 |
| SUL | 20 | 16 | 35.93 ± 0.707 | 1.97 | 36.98 ± 0.721 | 1.95 |
| | | 20 | 40.08 ± 0.693 | 1.73 | 40.09 ± 0.651 | 1.62 |
| | | 24 | 44.20 ± 0.861 | 1.95 | 44.71 ± 0.663 | 1.48 |

***Mean of three determinations**

**Assay of tablet formulation**

The assay of tablets for both methods was reported in table 4. The standard deviation of five replicate analysis for each method were found to be < 2.

**Table 4: Assay of tablets**

| Parameters | Multicomponent mode | | Dual wavelength method | |
|---|---|---|---|---|
| | PYR | SUL | PYR | SUL |
| Label claim (mg per tablet) | 25 | 500 | 25 | 500 |
| Drug content % ± SD* | 101.26 ± 0.82 | 99.43 ± 0.83 | 100.21 ± 1.12 | 99.72 ± 0.98 |
| % RSD | 0.81 | 0.83 | 1.12 | 0.98 |

***Mean of five determinations**

**CONCLUSION**

The obtained results from dual wavelength and multicomponent mode methods for simultaneous estimation of pyrimethamine and sulphadoxine indicate that the methods are simple, accurate and precise, hence can be used for routine analysis of commercially available drugs.

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