Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Research Article

Validated Stability Indicating Spectrophotometric Method for Estimation of Degradation Behavior of Pyrimethamine and Sulphadoxine in Tablet Formulation

Raghunath D. Wadulkar[1]*, T. M. Kalyankar[2], O. A. Khogre[2], K. Anitha[3]

Received on: 24-09-2016
Accepted on: 28-09-2016
Published on: 15-10-2016

Corresponding Author:
*** Raghunath D. Wadulkar,**
Shivlingeshwar College of Pharmacy,
Department of Pharmacology,
Almala, Tq: Ausa Dist: Latur.
Maharashtra, India.
Contact No: +91-9421604150

*Email Id- rwadulkar19@gmail.com

ABSTRACT

A new, simple, rapid and novel stability indicating spectrophotometric method has been developed and validated for estimation of degradation behavior of pyrimethamine and sulphadoxine in tablet formulation. For this, first order derivative method is used. The method involved measurement of absorbance at two wavelengths 273.8 nm and 262.5 nm of pyrimethamine and sulphadoxine respectively. This method obeys Beer's law in the employed concentration ranges of 1-6 µg/ml and 4-24 µg/ml for pyrimethamine and sulphadoxine respectively. Beer's law was obeyed in this concentration range with correlation coefficient of 0.997 and 0.998 for pyrimethamine and sulphadoxine respectively. The accuracy and precision of the method were determined and validated according to ICH guidelines. The method has good reproducibility with percent relative standard deviation (% RSD) less than two. The proposed method was validated for accuracy, precision, linearity, limits of detection (LOD), quantitation (LOQ) and reproducibility. The observations were found within the limits, stability study was performed for both drugs by using acid, base, neutral hydrolysis, oxidation, thermal and photolytic degradations which indicate the stability indicating property of the method. The proposed method is recommended for routine analysis work since it is rapid, simple, accurate, specific and sensitive.

Key-words: Sulphadoxine, Pyrimethamine, UV Spectrophotometric method, Validation, First order derivative.

Cite this article as:
Raghunath D. Wadulkar, T. M. Kalyankar, O. A. Khogre, K. Anitha, Validated Stability Indicating Spectrophotometric Method for Estimation of Degradation Behavior of Pyrimethamine and Sulphadoxine in Tablet Formulation, Asian Journal of Pharmaceutical Technology & Innovation, 04 (20); 16-25, 2016. www.asianpharmtech.com

[1]*Shivlingeshwar College of Pharmacy, Almala, Latur*
[2]*Department of Pharmaceutical Chemistry, School of Pharmacy, Swami Ramanand Teerth Marathwada University, Vishnupuri, Nanded-431606, Maharashtra, India.*
[3]*Department of Chemistry, Sri Krishnadevaraya University, Ananthapuramu-515003, Andhra Pradesh, India*

1. INTRODUCTION

Chemically Pyrimethamine (PYR) is a folic acid antagonists and used for protozoal infections. The chemical name for Pyrimethamine (Fig. 1 a) is 5-(4-chlorophenyl)-6-ethylpyrimidine-2, 4-diamine. Pyrimethamine is a medication used for protozoal infections. It is commonly used as an antimalarial drug. Pyrimethamine interferes with tetrahydrofolic acid synthesis from folic acid by inhibiting the enzyme dihydrofolate reductase (DHFR). Tetrahydrofolic acid is needed for DNA and RNA synthesis in many species, including protozoa.

Sulphadoxine (SPD) is N1-(5, 6-dimethoxypyrimidin-4-yl) sulphanilamide. Sulphadoxine (also spelled sulfadoxine) is an ultra-long-lasting sulfonamide. It is under the category of antimalarials, anti-Infective agents. Sulphadoxine (Fig. 1 b) competitively inhibits dihydropteroate synthase, interfering with folate synthesis. It acts by increasing oxygen in blood.[1-3]

Human malaria is caused by parasite of the genus *Plasmodium*. Four species are known to cause human malaria namely, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae*. Nevertheless, *P. falciparum* has been found to be the most lethal of all human malaria parasites. From decades, chloroquine was the most widely used treatment of malaria, but resistance has increased to such an extent that it has become ineffective in almost all malaria endemic countries. A fixed-dose combination of sulfadoxine and pyrimethamine, the usual successor to failing chloroquine, has been widely implemented in the last decade and is now one of the most widely used antimalarial treatments in the world. Sulfadoxine and pyrimethamine are active against the asexual erythrocytic stages of *Plasmodium falciparum*.[4-5]

In the literature survey it was found that pyrimethamine and sulphadoxine are estimated independently and in combination with other drugs by several HPLC and spectrometric methods.

This paper is in continuation with our work [21-26] where we studied spectrophotometric method for single or multicomponent drugs. However no references have been found for stability indicating assay method found for simultaneous determination of pyrimethamine and sulphadoxine even though this multi-ingredient's formulation is available in market.[6-16]

The aim of this work is to develop and validate stability indicating spectrophotometric method for estimation of degradation behavior of pyrimethamine and sulphadoxine in tablet formulation as per ICH guidelines[17-19] and to perform degradation studies on the drugs as per ICH guidelines using the proposed method.[20]

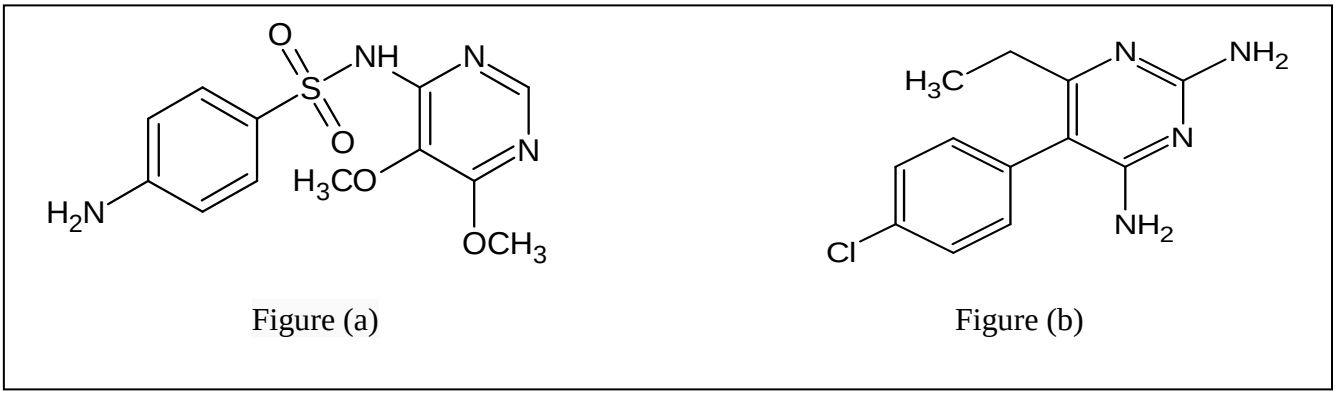

Figure (a) Figure (b)

Figure 1: Chemical structure of (a) sulphadoxine (b) pyrimethamine

2. MATERIALS AND METHODS

2.1 Apparatus and instruments

Spectrophotometric measurements were made on UV-visible double beam spectrophotometer (UV-1800, Shimadzu Corp., Japan) attached with computer operated software UV probe 2.0 with spectral bandwidth of 2 nm and 10 mm matched quartz cells were used for development of analytical method over the range of 200-400 nm. The drugs and chemicals were weighed on analytical digital weighing balance (AA – 2200, Anamed) and ultrasonic bath (HMG, India: CD-4820), hot air oven, UV-Chamber, Water bath were used during the study.

2.2 Chemicals and reagents

Standard bulk sample of sulphadoxine and pyrimethamine were pursued as a gift sample from Micro Lab. Ltd., Bangalore. Methanol (S. D. fine chemicals Ltd., India) is used as solvent and other chemicals were used as of AR grade which are sodium hydroxide, hydrochloric acid were obtained from RFCL, New Delhi. Hydrogen peroxide was obtained from Merck Ltd., Mumbai.

2.3 Marketed formulation

Marketed tablet formulation Reziz (pyrimethamine-25 mg, sulphadoxine-500 mg) tablets marketed by Shreya life sciences Ltd., Aurangabad was used as sample and purchased from local pharmacy.

2.4 Selection of common solvent

Methanol of analytical reagent grade was selected as a common solvent for developing spectral characteristic**s** of both drugs. The selection was made after assessing the solubility of both drugs in different solvents.

2.5 Preparation of standard solutions (100 µg/ml)

Standard stock solutions were prepared by accurately weighed 10 mg each of PYR and SPD and transferred to two separate 100 ml volumetric flasks and volume was made up with methanol. The stock solution was sonicated for 10 min for degassing and volume was made up to 100 mL with methanol.

3. First-order derivative spectrophotometric method

Working standard solutions of PYR (100 µg/ml) and SPD (100 µg/ml) were diluted with methanol to get concentration of 10 µg/ml for PYR and SPD respectively. These diluted solutions of PYR and SPD were scanned in spectrum mode between 400-200 nm using methanol as a blank. Then zero order spectrums of both the drugs were transformed mathematically into their individual first order derivative spectrum and first order derivative overlain of both the drugs were obtained in 400-200 nm which are shown in figure 2 (a, b, c).

3.1 Selection of analytical wavelength

For the development of method, the wavelength maxima of both the drugs were required. Appropriate dilutions were done for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. PYR and SPD showed absorbance maxima at 273.8 nm and 262.5 nm respectively from the overlain spectra by first order derivative spectrophotometric method in such a way that at zero crossing of one drug another drug showed substantial absorbance (zero crossing method).

3.2 Calibration curves for pyrimethamine and sulphadoxine

Appropriate aliquots of PYR and SPD working standard solutions were taken in different 10 mL volumetric flasks and diluted up to the mark with methanol to obtain final concentrations of 1, 2, 3, 4, 5, 6 µg/ml and 4, 8, 12, 16, 20, 24 µg/ml of PYR and SPD respectively. Spectra for the solutions were scanned between 400-200 nm. The absorbance of solutions was measured at 273.8 nm and 262.5 nm against methanol as blank, and the absorbance versus concentration was plotted and is shown in figure 3 (a, b) and results are mentioned in Table 2.

Table 2: Linear regression data for calibration curve of PYR and SPD

Name of the drug	Linearity range (µg/ml)	r^2	Slope	Intercept
PYR	1-6	0.997	0.027	0.004
SPD	4-24	0.998	0.065	0.008

3.3 Analysis of marketed tablet formulation

Accurately weighed 20 tablets of marketed formulation Reziz and average weight were found to be 602 mg then it was crushed to getting the fine powder. The 602 mg powder equivalent to 25 mg of PYR and 500 mg of SPD was weighed and dissolved in methanol. Which was then Sonicated for 20 min and filtered through Whatmann filter paper. From this sample solution by further dilution technique 1 µg/ml and 20 µg/ml concentrations of PYR and SPD obtained respectively and absorbance was measured at respective wavelengths. The analysis procedure was repeated six times and results are mentioned in Table 1.

Table 1: Analysis of tablet formulation

Sr. No.	Label claim (mg/tab)		Amount found (mg/tab)		% assay	
	PYR	SPD	PYR	SPD	PYR	SPD
1	25	500	24.85	494.85	99.40	98.97
2	25	500	24.81	493.7	99.25	98.74
3	25	500	24.88	495.8	99.55	99.16
4	25	500	24.86	497.45	99.47	99.49
5	25	500	24.94	496.65	99.77	99.33
6	25	500	24.91	497.9	99.65	99.58
				Mean*	99.51	99.21
				SD	0.1843	0.3195
				%RSD	0.1852	0.3221

** Indicates average of six determinations*

3.4 Analytical method validation

The proposed analytical method was validated with respect to parameters such as linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy and precision as per ICH guidelines.[17-19]

3.4.1 Linearity

Both drugs followed the Beer-Lambert's law in the range of 1-6 µg/ml and 4-24 µg/ml for PYR and SPD respectively. Absorbances for these solutions were measured at 273.8 nm and 262.5 nm for PYR and SPD respectively. Calibration curve are shown in figure 3; Regression coefficient (r^2) were found to be 0.997 and 0.998 for PYR and SPD respectively.

3.4.2 Precision

Precision of the method was verified by using stock solutions in the ratio of 1:20 containing 1 µg/ml of PYR and 20 µg/ml of SPD.

3.4.2.1 Repeatability (intra-assay precision)

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Six samples of the tablet formulations were analyzed for the repeatability study. The standard deviation and % RSD was calculated.

3.4.2.2 Intermediate precision

The intermediate precision of the method was evaluated by interday and intraday variation studies. In intraday studies, working solutions of sample were analyzed in triplicate for a day and percentage relative standard deviation (% RSD) was calculated. In the interday variation studies, working solution of sample were analyzed on three consecutive days and percentage relative standard deviation (% RSD) was calculated. The results of intermediate precision are given in Table 3.

3.4.3 Accuracy

To ascertain the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%) as per ICH guidelines. Known amount of pure PYR and SPD were added in preanalyzed powder of tablet formulation and analysis was carried out by proposed method for recovery at each level and % recovery, SD, % RSD was calculated. The results of recovery study are reported in Table 4 and 5.

3.4.4 LOD and LOQ

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal to noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and calculated with use of the following equations:

$$LOD = \frac{3.3\sigma}{S} \qquad LOQ = \frac{10\sigma}{S}$$

Where σ is the standard deviation of the peak areas of the drugs, taken as a measure of noise, and S is the slope of the corresponding calibration curve. The values are mentioned in Table 6.

3.5 Forced degradation studies

Stress degradation studies were performed to check the stability of the PYR and SPD on different conditions. The stress conditions applied for degradation study involved acid, base, neutral hydrolysis, thermal and photolytic degradation.[9] Standard stock solution of PYR 1 µg/ml and SPD 20 µg/ml was prepared in methanol. Summary of the forced degradation of PYR and SPD are mentioned in Table 7.

3.5.1 Acid hydrolysis

Stock solution 30 ml were prepared in 50 ml volumetric flask using methanol then added 10 ml of 0.1 M HCl and kept at room temperature (24±2 ^{0}C) for 12 hrs then neutralized with 10 ml of 0.1 M NaOH further (1 µg/ml for PYR and 20 µg/ml for SPD) dilutions was prepared and absorbance's were measured at 273.8 nm for PYR and 262.5 nm for SPD. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

3.5.2 Base Hydrolysis

Stock solution 30 ml were prepared in 50 ml volumetric flask using methanol then added 10 ml of 0.1 M NaOH and kept at room temperature (24±2 ^{0}C) for 12 hrs then neutralized with 10 ml of 0.1 M HCl further (1 µg/ml for PYR and 20 µg/ml for SPD) dilutions was prepared and absorbance's were measured at 273.8 nm for PYR and 262.5 nm for SPD. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

3.5.3 Neutral hydrolysis

Stock solution 40 ml were prepared in 50 ml volumetric flask using methanol then added 10 ml of water and kept at room temperature (24±2 ^{0}C) for 12 hrs further (1 µg/ml for PYR and 20 µg/ml for SPD) dilutions was prepared and absorbance's were measured at 273.8 nm for PYR and 262.5 nm for SPD. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

3.5.4 Oxidative degradation

Oxidative degradation was carried out at room temperature by treating solutions of 1 µg/ml for PYR and 20 µg/ml for SPD with 3% H_2O_2 for 6 hrs. Absorbance was measured in every 1 hr time interval at respective wavelengths. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

3.5.5 Photolytic degradation

Pure drugs were exposed to UV radiations for 6 hrs. The samples after exposure to light were diluted with methanol to get 1 µg/ml for PYR and 20 µg/ml for SPD and absorbance was measured at every 1 hr of time interval at 273.8 nm for PYR and 262.5 nm for SPD. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

3.5.6 Thermal degradation

Thermal degradation was carried out by exposing pure drugs to dry heat at 80ºC for 6h samples were withdraw at every 1 hr. The samples after exposure to heat were prepared and diluted with methanol to get 1 µg/ml PYR and 20 µg/ml of SPD and absorbance was measured at 273.8 nm for PYR and 262.5 nm for SPD respectively. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

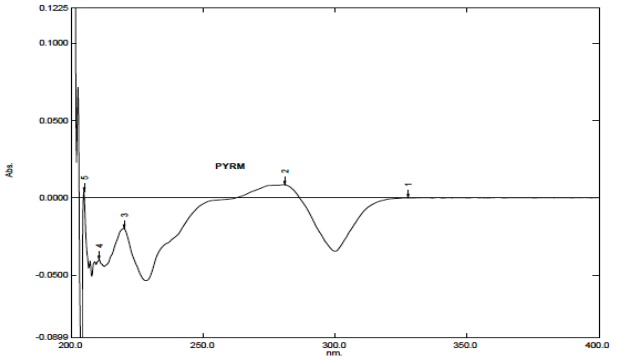

Figure (a)

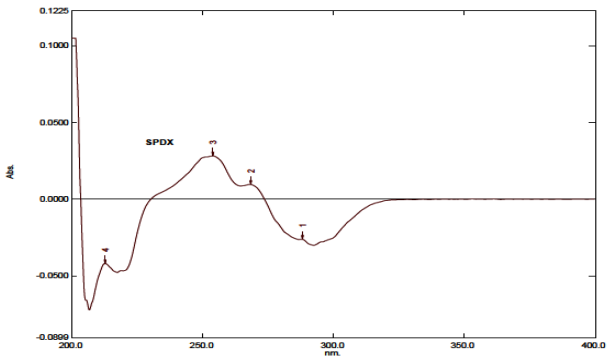

Figure (b)

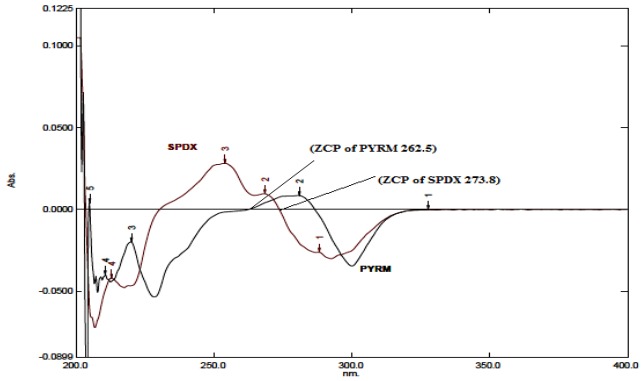

Figure (c)

Figure 2: 1st order derivative spectrum of (a) PYR (b) SPD (c) overlay of PYR and SPD

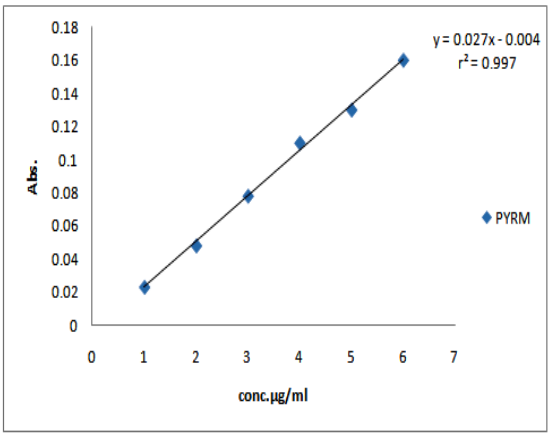

Figure (a) Pyrimethamine

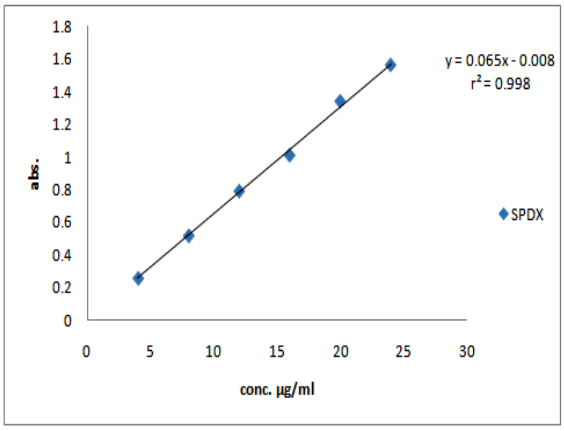

Figure (b) Sulphadoxine

Figure 3: (a) Calibration curve of PYR (b) Calibration curve of SPD

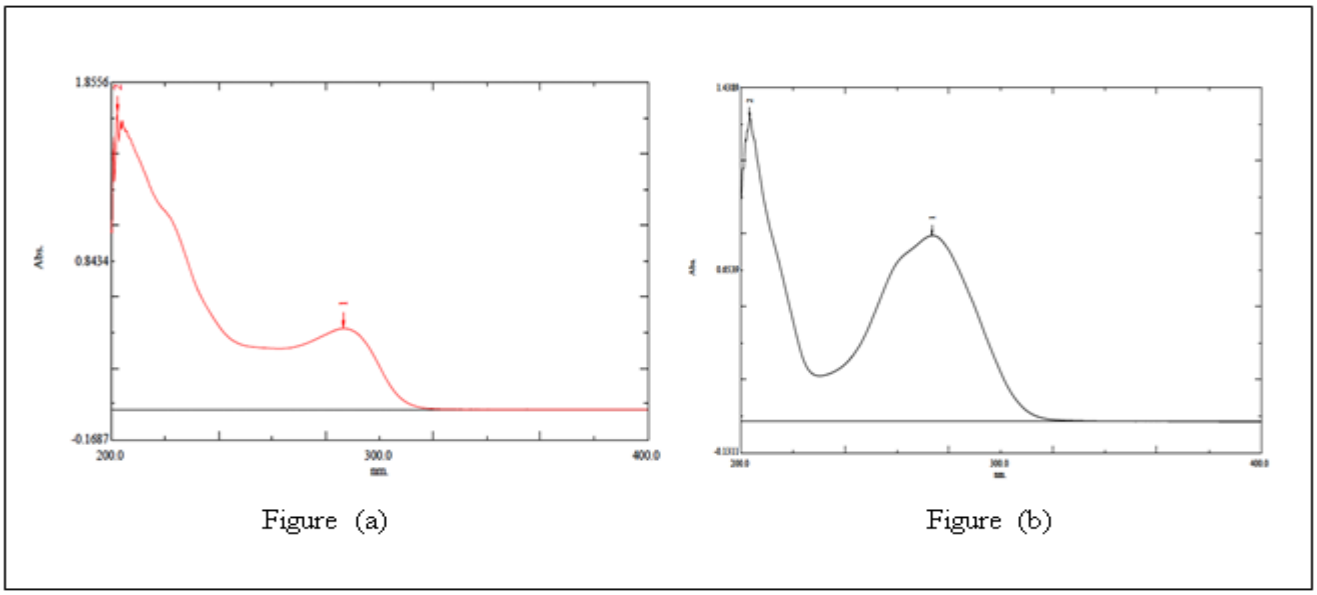

Figure 4: (a) Absorption spectrum of PYR (b) Absorption spectrum of SPD

4. RESULTS AND DISCUSSION

The validated stability indicating spectrophotometric method for estimation of degradation behavior of pyrimethamine and sulphadoxine in tablet formulation has been developed using methanol as solvent. PYR and SPD follow Beer's law in the employed concentration ranges of 1-6 µg/ml and 4-24 µg/ml. Commercial formulation containing PYR and SPD were analyzed by proposed method. Mean assay values in Reziz were found to be 99.51 and 99.21 for PYR and SPD respectively. The accuracy of method was determined by recovery studies. Pure PYR and SPD were added to the preanalyzed tablet powder at three different levels i.e. 80, 100 & 120% of label claims as per the ICH guidelines. Three replicate analyses were carried out at each level. The mean recovery was found to be 99.74%, 100.17%, 100.41% and 98.79%, 100.21%, 100.46% for PYR and SPD in Reziz samples respectively. It indicating that the method has required accuracy and there was no interference with API by excipients present in tablets. The RSD value is below 2% indicated that the method has required precision. The LOD and LOQ values of PYR and SPD at 273.8 nm and 262.5 nm were found to be 0.1210 and 0.3692 µg/ml and 0.0506 and 0.1535 µg/ml respectively.

Table 3: Results for intra-day and inter-day precision

Sr. No.	Interval of Time	Concentration (µg/ml)		% Recovery	
		PYR	**SPD**	**PYR**	**SPD**
I		1	20	99.40	98.97
II	Intra-day	1	20	99.25	98.74
III		1	20	99.55	99.16
			Mean*	99.40	98.95
			SD	0.1500	0.2103
			%RSD	0.1509	0.2125
I		1	20	99.47	99.49
II	Inter-day	1	20	99.77	99.33
III		1	20	99.65	99.58
			Mean*	99.63	99.46
			SD	0.1509	0.1266
			%RSD	0.1515	0.1273

** Indicates average of six determinations*

Table 4: Recovery study data

Level of Recovery	Amount present (mg)		Added concentration (mg)		Amount recovered (mg)		% Recovery	
	PYR	SPD	PYR	SPD	PYR	SPD	PYR	SPD
	25	500	20	400	44.82	895.41	99.62	99.49
80%	25	500	20	400	44.93	896.94	99.85	99.66
	25	500	20	400	44.89	875.16	99.77	97.24
	25	500	25	500	49.96	1000.8	99.92	100.08
100%	25	500	25	500	50.11	1002.5	100.22	100.25
	25	500	25	500	50.18	1003.3	100.37	100.33
	25	500	30	600	55.24	1105.5	100.44	100.50
120%	25	500	30	600	55.15	1102.75	100.29	100.25
	25	500	30	600	55.28	1107.26	100.52	100.66

Table 5: Statistical validation of recovery study data

Level of Recovery	% Mean recovery *		SD		% RSD	
	PYR	SPD	PYR	SPD	PYR	SPD
80%	99.74	98.79	0.1167	1.350	0.1170	1.367
100%	100.17	100.21	0.2291	0.1276	0.2287	0.1274
120%	100.41	100.46	0.1167	0.2066	0.1162	0.2056

** Indicates average of three determinations*

Table 6: LOD & LOQ

Name of the drug	LOD (µg/ml)	LOQ (µg/ml)
PYR	0.1210	0.3692
SPD	0.0506	0.1535

Table 7: Force degradation study data

Sr. no.	Condition (at room temp.)	% Degradation		% Assay	
		PYR	SPD	PYR	SPD
1	Acid hydrolysis (0.1 M HCl for 12 hrs)	5.22	9.14	94.78	90.86
2	Base hydrolysis (0.1 M NaOH for 12 hrs)	5.51	8.26	94.49	91.74
3	Neutral hydrolysis (In water for 12 hrs)	6.11	7.14	93.88	92.86
4	Oxidative degradation (3% H_2O_2 for 6 hrs)	4.67	6.22	95.33	93.78
5	Photolytic degradation (In UV chamber for 6 hrs)	6.25	6.58	93.75	93.42
6	Thermal degradation (80^0C for 6hrs)	7.13	8.81	92.87	91.19

5. CONCLUSION

Novel stability indicating spectrophotometric method was developed and validated for the estimation of PYR and SPD in their combined dosage form. The proposed method is simple, sensitive, accurate, and precise which requires less time with low cost for the analysis as compared to chromatographic method. Forced degradation studies were carried for PYR and SPD which can prove the extent of degradation in chosen experimental conditions. Hence, it can be successfully applied for routine estimation for PYR and SPD in quality control laboratories and pharmaceutical industries.

6. ACKNOWLEDGEMENT

Authors are thankful to Micro Lab. Ltd., Bangalore for providing the gift sample of the pure drug. The authors are also thankful to the Director, School of Pharmacy, S. R. T. M. University, Nanded, for providing research facilities.

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