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VALIDATED UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF SULFADOXINE AND PYRIMETHAMINE

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ABSTRACT

Method for simultaneous estimation of Sulfadoxine (SUL) and Pyrimethamine (PYR) was developed using alcoholic solubilization technique. Methanol was used as a solvent as both drugs are soluble in it. Methanol did not interfere in the spectroscopic determination of SUL and PYR having maximum absorbance at 268 nm and 282 nm respectively. SUL and PYR follow Beer-Lambert's law in range of 10-60 µg/ml and 1-25 µg/ml respectively. LOD and LOQ values of SUL and PYR were found to be 0.340 and 1.135 µg/ml and 0.003 and 0.090 µg/ml respectively. The proposed method is, therefore recommended for routine analysis since it is rapid, simple, accurate, precise, sensitive and specific.

KEYWORDS: Sulfadoxine, Pyrimethamine, Simultaneous Estimation, UV

INTRODUCTION

Antimalarial chemotherapy has been the primary option in the fight against malaria and over the years many drugs have been developed and used in the treatment of this disease. However, the burden of this disease is still very heavy partly due to the development of multi-drug resistant *Plasmodium falciparum* strains. The rate of increase in the resistance of the malaria parasite – *Plasmodium falciparum* – to antimalarial drugs in many parts of the world is becoming more disturbing.Because of the resistance problems associated with Chloroquine which was considered first-line therapy globally for many years, WHO convened an informal consultation on the use of antimalarial drugs. The potential value of malaria therapy using combinations of drugs was identified as a strategic and viable option in improving efficacy, and delaying development and selection of resistant parasites. [1] Sulfadoxine is 4-Amino-N-(5, 6-dimethoxpyrimidin-4-yl) benzene-1-sulfonamide. Sulfadoxine is a sulfa drug, often used in combination with Pyrimethamine to treat malaria. The sulfonamides are bacteriostatic antimicrobials that block the incorporation of p-aminobenzoic acid to form dihydropteroic acid. [2]Pyrimethamine is a diaminopyrimidine derivative with the specific chemical name 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine. Pyrimethamine inhibits the dihydrofolatereducates of plasmodia and blocks the biosynthesis of purines and pyrimidines, which are essential for DNA synthesis and cell multiplication. This leads to failure of nuclear division at the time of schizont formation in erythrocytes and liver. Structure of Sulfadoxine and Pyrimethamine are presented as below (Figure 1). [2]

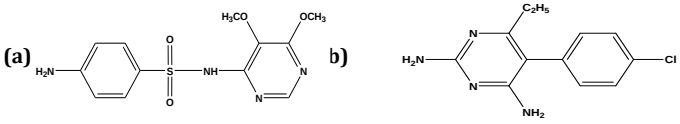

Figure 1. (a) Structure of Sulfadoxine and (b) Structure of Pyrimethamine

Working standard of Sulfadoxine and Pyremethamine were pursued as a gift sample from Cipla Ltd. and Macleoids Ltd. All chemicals and solvents of AR grade and were purchased from Qualigens fine Chemicals, Mumbai, India.UV- spectrophotometer UV-1800 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells were used for development analytical method over the range of 200-400 nm. Marketed formulation *Falcigo* tablet containing SUL 750 mg and PYR 37.5 mg was used as sample; purchased from local pharmacy. Calibrated glassware's were used throughout the work.

MATERIALS AND METHODS

Preparation of standard stock solutions

An accurately weighed quantity of about 10 mg of pure drug of SUL was dissolved in methanol and diluted to 100 ml to make stock solution of concentration 100 µg/ml. An accurately weighed quantity of about 5 mg of pure drug of PYR was dissolved in methanol and diluted to 100 ml to make stock solution of concentration 50 µg/ml.

Selection of analytical wavelengths

Appropriate dilutions were done for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. SUL and PYR showed absorbance maxima at 268 nm (Figure 2) and at 282 nm (Figure 3) respectively. Figure 4 represents the overlain spectra of both the drugs.

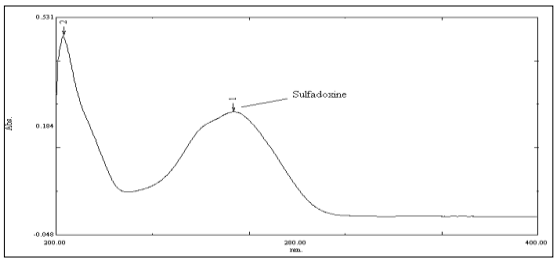

Figure 2. UV spectrum of SUL

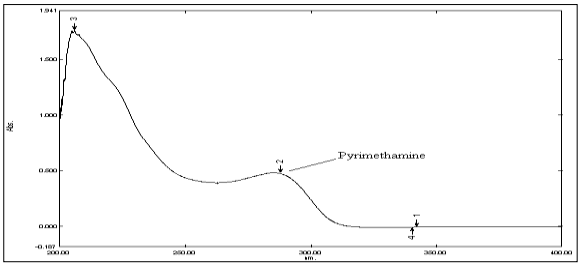

Figure 3. UV spectrum of PYR

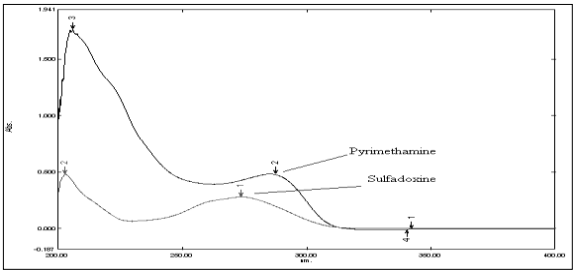

Figure 4. Overlain spectrum of the SUL and PYR

Selection of analytical concentration ranges

From the standard stock solution of SUL, appropriate aliquots were pipetted out into 10 ml volumetric flasks and dilutions were made with methanol to obtain working standard solutions of concentrations 10 - 60 µg/ml. Absorbance for these solutions were measured at 268 nm (Table

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1) and a calibration curve of absorbance against concentration was plotted (Figure 5).

Similarly, a series of standard solutions of concentration 1 - 25 µg/ ml were prepared for PYR and their absorbance were measured at 282 nm (Table 1). A standard calibration curve of absorbance against concentration was plotted (Figure 6). Both drugs followed the Beer-Lamberts law in the range of 10 - 60µg/ml and 1 - 25 µg/ml for SUL and PYR respectively. Table 2 summaries the optical characteristics of both the drugs.

Table 1. Standard calibration for SUL and PYR

Sr. No.	For Sulfadoxine		For Pyrimethamine	
	Conc. (µg/ml)	Abs. at 268 nm	Conc. (µg/ml)	Abs. at 282 nm
1.	10	0.294	1	0.043
2.	20	0.568	5	0.265
3.	30	0.902	10	0.530
4.	40	1.223	15	0.785
5.	50	1.534	20	1.043
6.	60	1.855	25	1.302

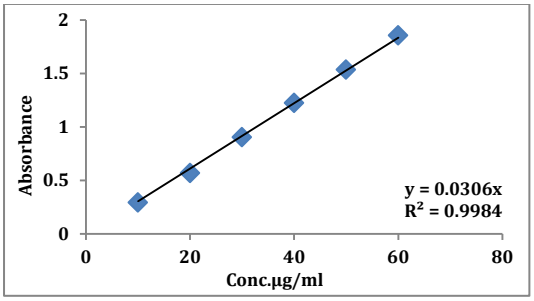

Figure 5. Calibration curve of SUL

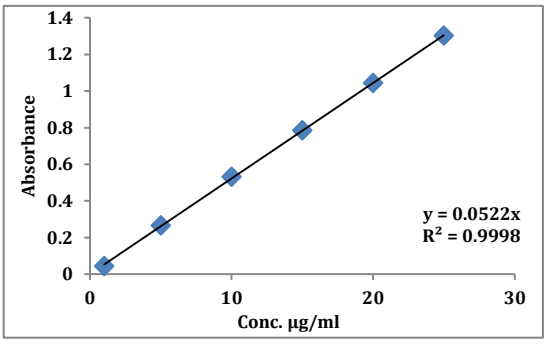

Figure 6. Calibration curve of PYR

Table 2: Optical characteristics and other parameters

Parameters	SUL	PYR
Working wavelength (nm)	268	282
Linearity range (µg/ml)	10-60	1-25
Molar absorptivity	29.4	53.0
Limit of detection (µg/ml)	0.340	0.003
Limit of quantitation (µg/ml)	1.135	0.0099
Y= mx + c		
Slope	0.030	0.522
Intercept	0.010	0.042
Regression Coefficient	0.999	0.999

Analysis of tablet formulation

For analysis of tablet formulation; first twenty tablets were weighed accurately; the average weight was determined and then triturated to a fine powder. A quantity equivalent to 750 mg of SUL and 37.5 mg of PYR was weighed and transferred to a 100 ml volumetric flask. Added 70 ml methanol the contents were sonicated for 20 min to dissolve the active ingredients and the volume was made up to 100 ml with methanol and filtered through Whatman filter paper no. 41 to give the stock solution containing 7500 µg/ml of SUL and 375 µg/ml of PYR. Various dilutions of the tablet stock solutions were scanned and the absorbances of these solutions were measured at 268 nm and 282 nm respectively and the concentrations of the two drugs in the sample solutions were determined by simultaneous equation. The analysis procedure was repeated six times. The results of marketed tablet formulation are given in Table 3.

RESULTS

Table 3. Results of marketed tablet formulation

Sr. No.	Label Claim (mg/tab)		Amount Found (mg/tab)		% of Label Claim	
	SUL	PYR	SUL	PYR	SUL	PYR
1	750	37.50	748.92	37.53	99.85	100.08
2	750	37.50	750.32	37.49	100.04	99.97
3	750	37.50	749.71	37.38	99.96	99.68
4	750	37.50	747.96	37.47	99.72	99.92
5	750	37.50	749.89	37.51	99.98	100.02
6	750	37.50	750.36	37.48	100.04	99.94
				Mean	99.93	99.89
				SD	0.1249	0.1241
				% RSD	0.1249	0.1242

Formulation: *Falcigo* (ZydusCadila Pharmaceutical Ltd, Ahemedabad)

Method Validation

Linearity

Both drugs followed the Beer-Lamberts law in the range of 10-60 µg/ml and 1-25 µg/ml for SUL and PYR respectively. Caliberation curve were shown in Figure 4 & 5; regression coefficient are 0.998 and 0.999 for SUL and PYR respectively.

Recovery study

Recovery studies were carried out at three levels i.e. 80, 100 and 120 % of the label claim of the Tablet formulation as per ICH guidelines. To perform recovery studies at 80 % of the test concentration, sample containing 750 mg of SUL and 37.5 mg PYR was weighed and transferred to a 100 ml volumetric flask. To it 600 mg of standard SUL and 30 mg of standard PYR was added, the mixture was mixed thoroughly. Added 70 ml methanol the contents were sonicated for 20 min with methanol to dissolve the active ingredients and the volume was made up to 100 ml with methanol and filtered through Whatman filter paper no. 41.

Similarly to perform recovery studies at 100 % of the test concentration, tablet powder containing 750 mg of SUL and 37.5 mg of PYR was weighed. To it, 750 mg of standard SUL and 37.5 mg of standard PYR was added and at 120 % level, 900 mg of standard SUL and 45 mg of standard PYR was added to the tablet powder equivalent to 750 mg of SUL and 37.5 mg of PYR. These contents were sonicated for 20 min with 70ml methanol to dissolve the active ingredients and the volume was made up to 100 ml with methanol and filtered through Whatman filter paper no. 41.
From the stock solutions prepared at each level suitable aliquots were pipetted out and diluted to 10 ml with methanol and were analysed as per the procedure for tablet formulations. The results of the recovery studies were also validated statistically. The results of recovery studies are given in Table 4.

Precision of method

Precision of the method was verified by using stock solutions in the ratio of 1:20 containing 1.5 µg/ml of PYR and 30 µg/ml of SUL. System repeatability was done by repeating the assay three times of six replicate dilutions of the same concentration after every two hours on the same day for intraday precision. Inter-day precision was carried out by performing the assay of six sample sets after 24 hours and 48 hours. The results of intermediate precision are given in Table 5.

LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) of the SUL and PYR were calculated using the standard deviation of responses (N) and slopes (S) of respective calibration curves using signal-to-noise ratio.

$$LOD = 3.3 \times N/S$$
$$LOQ = 10 \times N/S$$

Table 4. Results of recovery studies

Level of (%) Recovery	Amount present (mg/tab)		Amount of standard added(mg)		Total amount recovered (mg)		% Recovery*	
	SUL	PYR	SUL	PYR	SUL	PYR	SUL	PYR
80	750	37.5	600	30	1343.6	67.19	99.52	99.53
100	750	37.5	750	37.5	1493.70	74.61	99.57	99.48
120	750	37.5	900	45	1646.07	82.26	99.75	99.71
						Mean	99.61	99.57
						SD	0.0987	0.0981
						% RSD	0.0990	0.0985

*Each value is the mean of three observations

Table 5. Results of intermediate precision

Formulation	Parameter	Intra-day precision*	Inter-day precision*
SUL	% Mean	99.50	99.85
	SD	0.2458	0.1609
	RSD	0.2470	0.1611
PYR	% Mean	99.44	99.92
	SD	0.1401	0.0964
	RSD	0.1408	0.0964

*Each value is a mean of six observations.

Table 6. Results of LOD & LOQ

Parameters	SUL	PYR
Limit of detection (µg/ml)	0.340	0.003
Limit of quantitation (µg/ml)	1.135	0.090

DISCUSSION

The novel method for simultaneous estimation of SUL and PYR was developed using methanol as solvent. SUL and PYR follows Beer-Lambert's law in range of 10-60 µg/ml and 1-25 µg/ml shows SUL and PYR can be estimated in Methanol. Commercial formulation containing SUL and PYR were analyzed proposed method. Mean assay values in Falcigo were found to be 99.93±0.63 and 99.89±0.96 for SUL and PYR respectively.

The accuracy of method was determined by recovery studies. Pure SUL and PYR were added to the preanalyzed tablet powder at three different levels i.e. 80, 100, 120% of labeled claims as per the ICH guidelines. Three replicate analyses were carried out at each level. The mean recovery was found to be 99.61±0.51 % and 99.57±0.76 % for SUL and PYR in Falcigo samples respectively indicating that the method has required accuracy and there was no interference by excipients present in tablets. The RSD value below 2% indicated that the method has required precision. LOD and LOQ values at 268 and 282 were found to be 0.340 and 0.003 µg/ml and 1.135 and 0.0099 µg/ml respectively.

Thus, the developed method is simple, accurate and precise and can be used for routine analysis of SUL and PYR in pharmaceutical preparation.

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