

Spectrophotometric Methods For Simultaneous Determination of Sulphadoxine and Pyrimethamine in Two Component Tablet Formulations

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Simultaneous determination of active ingredients in multicomponent pharmaceutical products normally requires the use of separation techniques, such as HPLC or GC, followed by their quantitation. Presented here are two analytical methods, *i.e.*, derivative spectroscopy and multi-wavelength spectroscopy, that does not require prior separation for simultaneous determination of sulphadoxine and pyrimethamine in two component tablet formulation. Shimadzu UV 160A spectrophotometer, capable of multicomponent analysis, was used for quantitation. Determinations were made in 0.1 N methanolic hydrochloric acid. Zero crossing point technique of measurement is used in derivative spectroscopy, whereas multiwavelength spectroscopic method is based on principle of overdetermination. Recordings of absorbances of standard solutions at 240 nm, 245 nm and 250 nm were processed by means of matrix equations and results for sample solutions were obtained. Beer's law is obeyed in the concentration range of 0-30 mcg/mL of sulphadoxine and 0-10 mcg/mL of pyrimethamine. The utility of developed methods has been demonstrated by analysis of commercial formulation containing both the drugs. The methods have been validated statistically and were found to be satisfactory.

INTRODUCTION

Sulphadoxine is ultra long acting sulphonamide, whereas pyrimethamine is inhibitor of enzyme dihydrofolate reductase in plasmodium. Sulphadoxine in combination with pyrimethamine is used for the treatment of malaria, particularly caused by chloroquine resistant strains of *P. falciparum*¹.

IP², BP³ and USP⁴ describe nitrite titration method for analysis of sulphadoxine in powder form. For its tablet formulation containing pyrimethamine, IP and USP describe HPLC method. Various methods reported in literature for the analysis of sulphadoxine in dosage and biological fluids include spectrophotometry⁵⁻⁷ and HPLC⁸⁻¹⁰. Pyrimethamine is official in IP¹¹, BP¹² and USP¹³. Non-aqueous titrimetric method has been described in IP, BP and USP for the analysis of pyrimethamine in powder form, whereas spectrophotometric method has been described in USP for tablet formulation containing pyrimethamine. Spectrophotometry^{14, 15}, HPLC¹⁶ and Florimetric¹⁷ methods have been reported in literature for the analysis of pyrimethamine in dosage forms. The present paper describes two simple methods for simultaneous determination of sulphadoxine and pyrimethamine in combined dosage forms.

EXPERIMENTAL

Shimadzu (Model UV 160A) instrument was used for spectral measurement using 10 mm matched quartz cells. Instrumental parameters chosen were: spectral bandpass: 2 nm, scan speed: 480 nm/min, wavelength range: 300–200 nm and wavelength sampling interval: 0.1 nm. Samples of sulphadoxine (IP) and pyrimethamine (IP) were procured from Lupin Laboratories Limited. Methanol (Qualigens, Spectroscopic grade) and hydrochloric acid (Ranbaxy, AR grade) were used in the present work.

Method I: Employing Derivative Spectrophotometry

Overlay spectrum of sulphadoxine (25 mcg/mL) and pyrimethamine (10 mcg/mL) is shown in Fig. 1 and first order derivative spectrum ($\Delta\lambda = 7.2$ nm) of both the drugs is shown in Fig. 2.

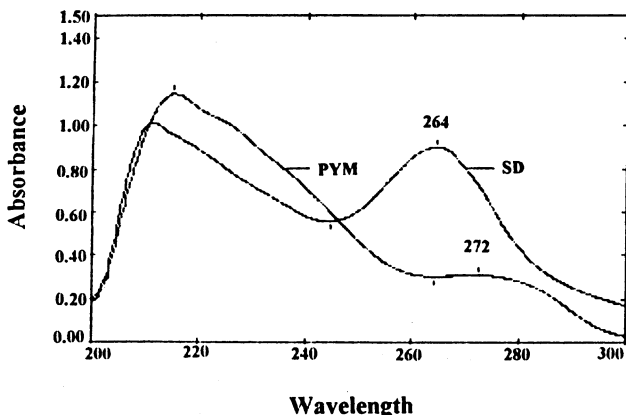


Fig. 1 Overlay spectrum of sulphadoxine (SD) and pyrimethamine (PYM)

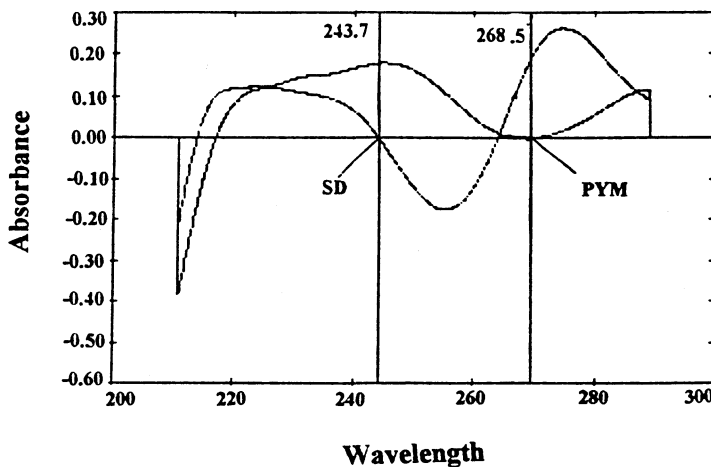


Fig. 2 First order derivative spectrum of sulphadoxine (SD) and pyrimethamine (PYM)

From first derivative spectrum of both the drugs, it is evident that sulphadoxine and pyrimethamine show zero absorbances at 243.7 and 268.5 nm respectively. As at the zero crossing point on the first derivative spectrum of one drug, other drug shows substantial absorbance; these two wavelengths can be employed for estimation of pyrimethamine and sulphadoxine respectively.

Standard stock solutions of sulphadoxine (100 mcg/mL) and pyrimethamine (10 mcg/mL) were prepared separately in 0.1 N methanolic hydrochloric acid. By appropriate dilutions of standard stock solutions, two series of mixed standard solutions were prepared: first series containing sulphadoxine and pyrimethamine in the concentration ratio of 5 : 1, 10 : 1, 15 : 1, 20 : 1 and 25 : 1 (mcg/mL) and the second series containing sulphadoxine and pyrimethamine in the concentration ratio of 20 : 1, 20 : 2, 20 : 3, 20 : 4 and 20 : 5 mcg/mL. Mixed standard solutions from both the series were scanned in the range of 300 to 200 nm and first derivative amplitudes at 243.7 nm (for first series) and 268.5 nm (for second series) were recorded. These spectral amplitudes were used to prepare calibration curves for sulphadoxine and pyrimethamine. Coefficients of correlation for sulphadoxine and pyrimethamine were 0.9997 and 0.9996 respectively.

Analysis of Commercial Formulations

Twenty tablets were ground to fine powder. An accurately weighed quantity of powdered tablets, equivalent to 20 mg of sulphadoxine, was transferred into a 100 mL volumetric flask. The powder was dissolved in about 75 mL of 0.1 N methanolic hydrochloric acid by intermittent shaking and volume was made up to 100 mL. The solution was filtered through Whatman filter paper no. 41 (stock solution).

TABLE-1
ANALYSIS OF COMMERCIAL FORMULATIONS

Formulation	Label claim mg/tab	Sulphadoxine				Pyrimethamine				
		Method I		Method II		Method I		Method II		
		% Estd.* (±SD)	SE	% Estd.* (±SD)	SE	% Estd.* (±SD)	SE	% Estd.* (±SD)	SE	
I	500	100.02 (±1.10)	0.45	99.37 (±0.84)	0.34	25	100.08 (±1.31)	0.53	100.28 (±0.94)	0.38
II	500	99.96 (±1.14)	0.47	100.92 (±1.04)	0.42	25	100.57 (±1.49)	0.61	100.76 (±1.40)	0.57
III	500	99.86 (±0.97)	0.39	99.28 (±0.95)	0.39	25	99.55 (±1.13)	0.46	100.30 (±1.22)	0.50

*Average of six determinations; SD: Standard Deviation, SE: Standard Error.

Stock solution (1 mL) was further diluted to obtain solution containing 20 mcg/mL of sulphadoxine. First derivative amplitudes of the resulting solution at 243.7 and 268.5 nm were recorded, and quantity of sulphadoxine and pyrimethamine present in the sample solution was obtained from the calibration curves plotted. The results obtained by repeating the procedure six times each with different formulations have been validated statistically (Table-1). The

recovery studies conducted by addition of different amounts of pure drug(s) to a pre-analysed tablet sample solution were also satisfactory (Table-2).

TABLE-2
RESULTS OF RECOVERY STUDIES

Drug	Concentration of added drug in final dilution (mcg/mL)	% Recovery	
		Method I	Method II
Sulphadoxine	2	98.40	100.84
	4	101.70	100.60
	6	99.46	99.70
Pyrimethamine	2	99.76	99.36
	4	98.83	99.13
	6	102.13	101.30

Method II: Multiwavelength Spectroscopy

From the overlay spectrum of sulphadoxine and pyrimethamine (Fig. 1), wavelengths that could be utilized for multiwavelength spectroscopy were 240, 245 and 250 nm, along with use of six mixed standards. These three sampling wavelengths were selected keeping in view the molar absorptivity coefficients of sulphadoxine and pyrimethamine and quantity of both the drugs in the marketed formulation. Six mixed standard solutions containing sulphadoxine and pyrimethamine in the concentration ratio of 0 : 10, 25 : 0, 5 : 2, 10 : 4, 15 : 6 and 20 : 8 (mcg/mL) were prepared in 0.1 N methanolic hydrochloric acid. All the mixed standard solutions were scanned over the range of 300–200 nm in multicomponent mode using the sampling wavelengths as mentioned above. Recordings of absorbances of the mixed standard solutions were processed by means of matrix equations and then correlated to determine the concentration of both the drugs in tablet sample solution, prepared as described under method-I. Stock solution (1 mL) was taken in 10 mL volumetric flask. To it, 1 mL standard pyrimethamine solution (10 mcg/mL) was added and volume was made upto the mark. Spectrophotometric analysis of the resulting solution was carried out using the multicomponent mode of the instrument. The results of analysis and recovery studies were found to be satisfactory (Tables 1 and 2).

RESULTS AND DISCUSSION

Both the proposed methods were found to be simple and rapid for routine analysis of sulphadoxine and pyrimethamine in combined dosage forms.

The first method, derivative spectroscopic method, requires spectral data processing and hence can only be applied on recording spectrophotometers. It is based on zero crossing point technique of measurement. First derivative spectrophotometry was employed considering the spectral responses and sensitivity of instrument used. The values of standard deviation were satisfactory, and recovery studies close to 100% were indicative of the accuracy of the method. Higher value of standard deviation for pyrimethamine is attributed to its low amount in the formulation. Selection of particular mixed standards to prepare the

calibration curves for sulphadoxine and pyrimethamine was done, keeping in view the quantity of sulphadoxine and pyrimethamine present in formulation and concentration obedience limits.

The second method, the multiwavelength spectroscopy is based on the principle of overdetermination. In this method, the instrument is preprogrammed to collect and compile the spectral data from the scan of standards and produces the results by matrix calculations. Sampling wavelengths 240, 245 and 250 nm were selected keeping in view the molar absorptivity coefficients of sulphadoxine and pyrimethamine. Standard deviation values for both the components were satisfactory, and recovery studies were close to 100%, indicating the reproducibility and accuracy of the method.

In multiwavelength spectroscopy, standard but known amount of pyrimethamine was added to the solution being analyzed in order to increase its absorbance contribution, which improves accuracy of estimation.

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