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Research Article

VALIDATED UV SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF AZITHROMYCIN AND PREDNISOLONE

DEEPAK MALHOTRA, ANUPAMA KALIA*, SONIA TOMAR

Department of Pharmaceutical Sciences, Lovely School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India.
Email: anukalia@ymail.com

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ABSTRACT

Azithromycin (AZI) is a semi-synthetic macrolide antibiotic drug, effective against a wide variety of bacteria. It is primarily used to treat the bacterial infections associated with weaker immune system. Prednisolone (PRE) is a synthetic corticosteroid, used for suppressing the immune system and inflammation. When used in combination, both the drugs are very much effective in the management of inflammatory conditions or diseases in which the immune system plays an important role. The present study describes a simple, accurate, reproducible and precise UV Spectrophotometric method for the simultaneous estimation of AZI and PRE in pH 6.8 Phosphate buffer. The absorbance maximum (λ_{max}) for AZI and PRE were found to be 298.6nm and 245nm. The method was validated for different parameters such as sandell's sensitivity, molar absorptivity, accuracy, precision, ruggedness, robustness, detection limit, quantification limit, etc (as per the ICH guidelines). The relative standard deviation (RSD) in case of accuracy, precision, ruggedness and robustness was less than 2.0% proving that method was highly accurate, precise and robust. This method can be used for the determination of AZI and PRE in pharmaceutical formulations without interference of the excipients.

Keywords: UV estimation, Azithromycin, Prednisolone, pH 6.8 Phosphate buffer, λmax, Validation.

INTRODUCTION

Azithromycin (AZI; fig. 1) is a broad spectrum semi-synthetic marcolide antibiotic, belonging to a new sub-class of macrolide antibiotics called azalides. It is used in the treatment of various bacterial infections, most often those causing middle ear infections, strep throat, pneumonia, typhoid, and sinusitis and various skin diseases [1-2]. Prednisolone (PRE; fig. 2) is a synthetic corticosteroid drug that is particularly effective as an immunosuppressant drug. It is used in the treatment of wide range of inflammatory and auto-immune conditions [2,3]. The combination of both the drugs would be beneficial for the treatment of various diseases associated with bacterial infection as well as altered immune system. In order to formulate combination of both the drugs, the primary requirement is the simultaneous estimation of both the drugs. Extensive literature survey suggested that a formulation containing these two drugs in combination has not been reported so far and hence the method of analysis is also not available. The present study is aimed to develop a selective, precise, accurate and reliable UV method for determination of AZI and PRE.

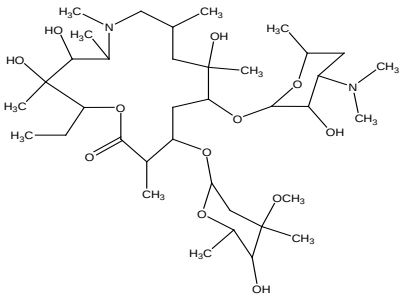

Fig. 1: Chemical structure of Azithromycin

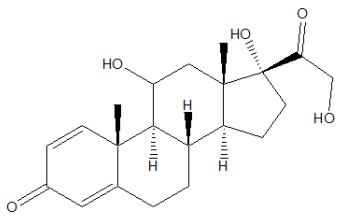

Fig. 2: Chemical structure of Prednisolone

MATERIALS AND METHODS

Instruments and reagents

Shimadzu UV/VIS double beam spectrophotometer (model 1800) with 1 cm matched quartz cells was used for all spectral measurements. UV probe version 2.33 software was used for the study. For robustness study, Systronics 2203 was used. Both these instruments have an automatic wavelength accuracy of 0.1 nm. AZI was provided as a gift sample by overseas pharmaceuticals, Phillaur, India. Methanol was purchased from Rankem, New Delhi, India. Hydrochloric acid, Starch, Hydroxy Propyl Methyl Cellulose (HPMC) and Lactose were procured from loba Chemie, Mumbai, India**.** All the reagents were of analytical grade.

Determination of absorption maxima of AZI and PRE in pH 6.8 phosphate buffer [4]

Accurately weighed 10 mg of AZI was transferred to a 10 ml volumetric flask and volume was made up with the 0.1N HCl (used as a cosolvent as AZI is not completely soluble in pH 6.8 phosphate buffer) to get a solution of concentration 1000 µg/ml. 1 ml of stock solution was diluted with pH 6.8 phosphate buffer up to 10 ml to get a concentration of 100 µg/ml and then further dilutions were made to obtain the concentration range of 10-45 µg/ml using pH 6.8 phosphate buffer. Solution of PRE was prepared in methanol (used as cosolvent) in a similar way to obtain the concentration range of 10-90 µg/ml by diluting with pH 6.8 phosphate buffer. Both the solutions were scanned in the spectrum mode over the range of 200-400 nm. AZI showed an absorbance peak at 298 nm, whereas PRE at 245 nm. The spectra also showed two isoabsorptive points at 298 nm and 245 nm respectively (Fig. 3).

Simultaneous vierodt's estimation method equation

The absorbance of sample solutions of AZI and PRE were measured at 298 nm and 245 nm respectively. The results were calculated by the following formula using Vierodt's method [5]

A1 = ax1 Cx + ax2 Cy at 298 nm
A2 = ay1 Cx + ay2 Cy at 245 nm

Where,

A1 and A2 are absorbance of diluted mixture of drugs at 298 nm and 245 nm respectively, Cx and Cy are the concentration of AZI and PRE respectively (µg/ml), ax1 and ax2 are absorptivities of AZI at 298 nm and 245 nm respectively, ay1 and ay2 are absorptivities of PRE at 298 nm and 245 nm respectively.

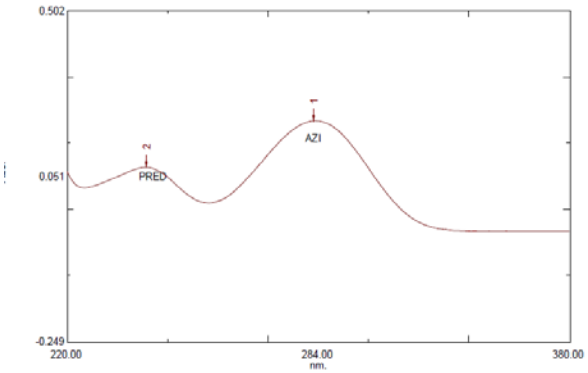

Fig. 3: Spectrum peaks for λ_{max} of AZI-PRE mixture on wavelength scan of 200- 400 nm

Analytical method validation

The method was validated for different parameters like linearity, specificity, selectivity, accuracy, precision, robustness, ruggedness, limit of quantification (LOQ) and limit of detection (LOD) [6-8]

Linearity

Linearity was checked by calculating regression coefficient [9].

Specificity and selectivity

Specificity and selectivity of the selected method was determined by preparing 10µg/ml of AZI and PRE solution in pH 6.8 Phosphate buffer along with and without common excipients (Lactose, Hydroxypropyl methylcellulose and starch) separately. All the solutions were scanned from 200-400 nm at fast speed and analyzed for any change in absorbance and percentage drug recovery at respective wavelengths.

Accuracy

The accuracy of the method was determined by calculating percentage drug recovery of AZI and PRE at a concentration of 10 µg/ml (n=6) [9].

Precision

The precision of proposed method was determined by varying the practical conditions. Inter-day, intra-day and inter-analyst variations were studied to determine the intermediate precision of proposed analytical method. Drug concentrations of AZI-PRE mixture was prepared at three different times in a day and studied for intra-day variation. The same procedure was followed for six days in order to study inter-day variations. Developed method was performed by three different analysts for inter analyst studies and by same analyst at six different times for intra analyst studies. The percent relative standard deviation (% RSD) of prepared concentrations was analyzed for precision studies [9].

Limit of detection and Limit of quantification

Limit of detection (LOD) and Limit of quantification (LOQ) of AZI-PRE mixture by proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S respectively. Where, σ is standard deviation of y-intercept of regression equation and S is the slope of the calibration curve [8-9].

Ruggedness and Robustness

Ruggedness and robustness was evaluated by analyzing AZI-PRE mixture under variable experimental conditions (a) Sample solution containing 10µg/ml was assayed for six times prepared from different stock solution and the percentage recovery was checked in each case. (b) Sample solution containing 10µg/ml was analyzed by changing the UV apparatus (Apparatus 1: Shimadzu-1800; Apparatus 2: Systronics 2300) to check the inter-instrument variation in the absorbance. (c) Robustness in the form of stability of AZI-PRE in prepared solution was also checked at room temperature. The concentration of drug in solvent was checked at time of preparation and at the interval of twenty-four hours [9].

RESULTS AND DISCUSSION

Standard calibration curve

The UV scan of standard solution of AZI between 200-400 nm gives the absorption maxima at 298 nm and PRE at 245.3 nm as shown in fig. 3. The regression coefficient for calibration curve of AZI in pH 6.8 Phosphate buffer was found to be 0.998 with regression equation of Y_{298nm} = 0.021X (fig. 4). The regression coefficient for calibration curve of PRE in pH 6.8 Phosphate buffer was found to be 0.999 with regression equation of Y_{245nm} = 0.010X (fig. 5). The optical and regression characteristics are given in table 1. The concentrations of AZI and PRE calculated by Vierodt's method are clear from table 2.

Table 1: Regression and optical characteristics of AZI-PRE in UV estimation method

Parameter	AZI	PRE
λ_{max} (nm)	298	245
Beer's law limit (µg/ml)	5-45	10-90
Regression coefficient	0.998	0.999
Regression equation	Y_{298}=0.021X	Y_{298}=0.010X
Slope	0.021	0.010

Table 2: Concentration of AZI-PRE obtained from Vierodt's method

Drug	Concentration taken (µg/ml)	Concentration obtained (µg/ml)
AZI (C_X)	10	9.818
PRE (C_Y)	10	9.101

Analytical method validation

Linearity

The linearity was found in the range of 5-45 µg/ml for AZI and 10-90 µg/ml for PRE, supported by high regression coefficient of 0.998 and 0.999 respectively as shown in fig. 4 and fig. 5. Overlaid spectra of AZI-PRE mixture from concentration 10-40 µg/ml is shown in fig. 6.

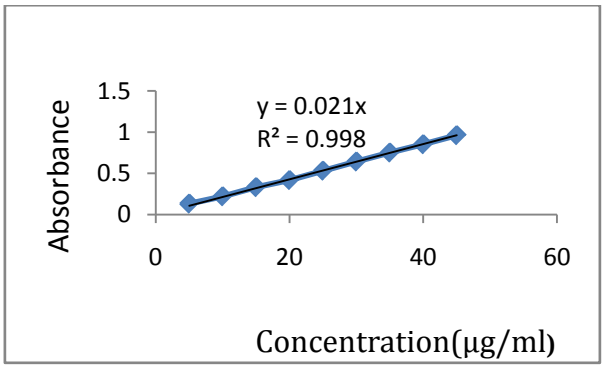

Fig. 4: Standard calibration curve of AZI in pH 6.8 Phosphate buffer

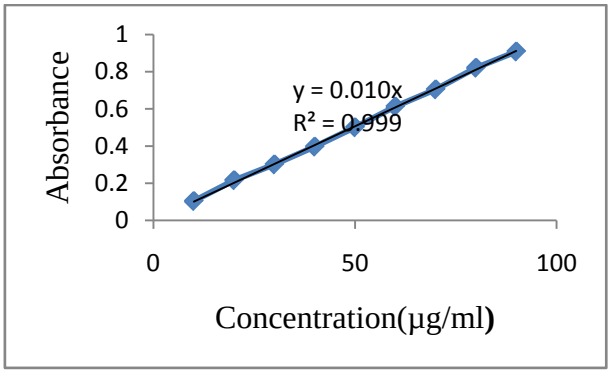

Fig. 5: Standard calibration curve of PRE in pH 6.8 Phosphate buffer

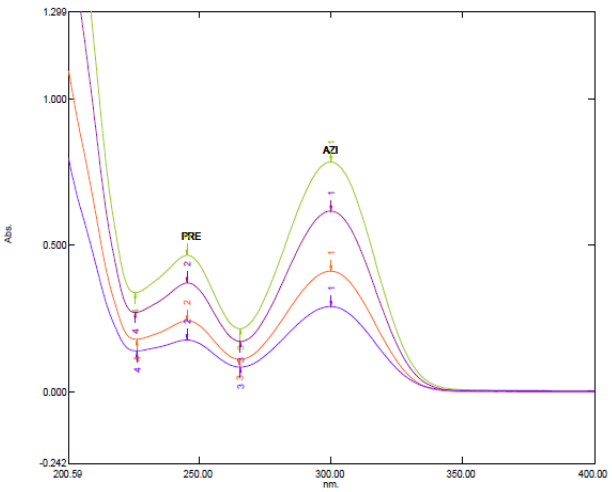

Fig. 6: Overlaid spectrum of AZI-PRE Mixture in the range of 10-40µg/ml on wavelength scan of 200-400nm

Specificity

The UV-Spectrum of AZI and PRE showed no change in the presence of common excipients used for the formulation. Absorption spectrum of pure drug sample was matching with the sample mixed with excipients. Table 3 shows the values for specificity studies of AZI-PRE in pH 6.8 Phosphate buffer. The excellent percentage recovery of AZI-PRE shows that there is no effect of excipients on the absorbance.

Accuracy

The accuracy of the method was checked by determining the percentage recovery values. Percentage drug recovery (± RSD) of above concentrations of AZI and PRE are shown in table 4. In all the cases RSD was not more than 2% depicting the accuracy of the developed method.

Precision

The precision of the developed method was determined by studying the repeatability of the developed method. In this, the percentage recovery of AZI and PRE was calculated by inter-day, intra-day, inter-analyst and intra analyst conditions over a short interval of time. Results of percentage drug recovery obtained from intra-day studies, inter-day studies and inter-analyst studies are shown in table 5. From obtained data, it was found that in all the precision studies, RSD was not more than 2 indicating that developed method has excellent repeatability.

Limit of Detection and Limit of Quantification

In case of AZI, LOD and LOQ was calculated to be 0.240 µg/ml and 0.728µg/ml respectively, while in case of PRE, LOD and LOQ was calculated to be 0.660µg/ml and 2.000µg/ml respectively.

Robustness

Robustness of the selected method was checked by inter-stock solution, inter-instrument, and by calculating percentage drug recovery after twenty-four hours of preparation of stock solution (Table 6). Overlaid spectra of stock solution at zero and twenty-four hours (fig. 7) showed no change in absorbance and λ_{max} of the solution. Further, data showed that in all the above cases, RSD was found to be less than 2 confirming the robustness of the developed method.

Table 3: Results of specificity and selectivity studies of applied method of UV estimation of AZI-PRE mixture.

	Excipient	Amount taken: Excipient added	% Drug recovery ± RSD AZI	% Drug recovery ± RSD PRE
Conc. of drug taken = 10µg/ml (N=6)	--	1:0	102.539±1.758	99.333±1.537
	Lactose	1:1	102.690±1.873	99.333±1.537
	HPMC	1:1	104.444±1.147	99.666±1.532
	Starch	1:1	97.936±1.965	102.333±1.492

Table 4: Results of accuracy studies of applied method of UV estimation of AZI-PRE mixture.

Accuracy of recovery studies			
(N=6)	**Conc. of drug taken (µg/ml) (µg/ml)**	**% Drug recovery ± RSD AZI**	**% Drug recovery ± RSD PRE**
	10	100±0.824	97±1.030

Table 5: Results of precision studies of applied method of UV estimation of AZI-PRE mixture

A.	**Intra-day studies**		
(N=3)	**Conc. of drug taken (µg/ml)**	**Observed Conc. ± RSD AZI**	**% Drug recovery ± RSD PRE**
	10	104.440±1.140	97.330±1.561
B.	**Inter-day studies**		
(N=6)	**Conc. of drug taken (µg/ml)**	**Observed Conc. ± SD AZI**	**Observed Conc. ± SD PRE**
	10	103.650±1.856	97.333±1.560
C.	**Inter-analyst studies**		
(N=3)	**Conc. of drug taken (µg/ml)**	**Observed Conc. ± SD AZI**	**Observed Conc. ± SD PRE**
	10	104.603±1.314	100.600±1.510

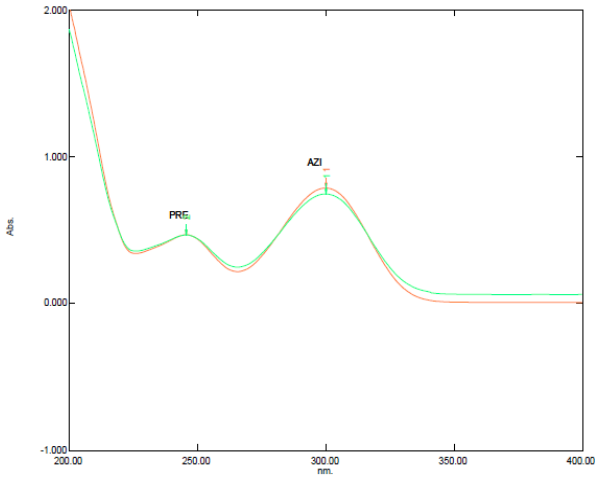

Fig. 7: Overlaid spectrum of AZI: PRE mixture (40 µg/m both) showing absorbance at zero and after twenty-four hours

Table 6: Results of robustness studies of applied method of UV estimation of AZI-PRE mixture.

A.	**Robustness of Inter-stock solution**		
	Conc. of drug taken (µg/ml)	**Observed Conc. ± SD**	**Observed Conc. ± SD**
(N=6)		**AZI**	**PRE**
	10	101.904±1.321	97±1.458
B.	**Robustness of Inter-instrument**		
	Conc. of drug taken (µg/ml)	**Observed Conc. ± SD**	**Observed Conc. ± SD**
(N=2)		**AZI**	**PRE**
	10	101.904±1.618	97.333±1.570
C.	**Robustness of stability of solution**		
	Conc. of drug taken (µg/ml)	**Observed Conc.± SD**	**Observed Conc. ± SD**
(N=6)		**AZI**	**PRE**
	40	103.091. ±0.326	99.500±0.710

CONCLUSION

A simple, rapid, accurate and précised method was developed for simultaneous UV determination of Azithromycin and Prednisolone. The method was validated and demonstrated a wide linear range, a good precision accuracy and specificity. The proposed method is a simple and rapid procedure. The method is sufficiently sensitive to measure Azithromycin and Prednisolone simultaneously.

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