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Dual Wavelength Spectrophotometric Method for Simultaneous Estimation of Ofloxacin and Cefpodoxime Proxetil in Tablet Dosage Form

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and cost effective dual wavelength spectrophotometric method for the simultaneous determination of ofloxacin and cefpodoxime proxetil in combined tablet dosage form. The utility of dual wavelength data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The method was based on determination of ofloxacin at the absorbance difference between 224 nm and 247.4 nm and cefpodoxime at the absorbance difference between 278.2 nm and 320 nm. The linearity was obtained in the concentration range of 2-12 µg/ml and 4-24 µg/ml for ofloxacin and cefpodoxime proxetil respectively. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found. The suitability of these methods for the quantitative determination of ofloxacin and cefpodoxime proxetil was proved by validation. The proposed methods were found to be simple and sensitive for the routine quality control application of ofloxacin and cefpodoxime proxetil in pharmaceutical tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: *Cefpodoxime proxetil, Dual wavelength spectrophotometric method, Ofloxacin, Validation.*
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INTRODUCTION

Ofloxacin (OFLO) is chemically 9-fluro-2-3 dihydro-3-methyl-10- (4-methyl 1-piperazinyl) - 7-oxo-7H- pyrido [1, 2, 3-de] 1, 4 benzoxazine-6-carboxylic acid[1] (Figure 1) is a fluoroquinolone antibacterial used in the treatment of chalmydia or chlamydophila infections including nongonococcal urethritis and in mycobacterial infections such as leprosy[2]. It is official in IP, BP and USP. IP[3], BP[4] and USP[5] describe potentiometry method for its estimation. Literature survey reveals spectrofluorimetric[6-7], HPLC[8-9] and chemiluminescence[10] methods for determination of OFLO in pharmaceutical dosage forms as well as in biological fluids. Literature survey also reveals spectophotometric[11], RP-HPLC[12] and HPTLC[12] methods for determination of OFLO with other drugs. Cefpodoxime proxetil (CEFPO) is chemically 1-(isopropoxy carbonyloxy) ethyl(6R,7R)-7-[2-(2-amino-4-thiazolyl)-(z)-2-(methoxyimino)acetamido] -3-methoxymethyl-3-cephem-4-carboxylate[13]

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(Fig.2) is a third generation cephalosporin antibiotic used for infections of the respiratory tract, urinary tract and skin and soft tissues[14]. Cefpodoxime proxetil is official in IP and USP. IP[15] and USP[16] describe liquid chromatography method for its estimation. Literature survey reveals HPTLC[17] method for the determination of CEFPO. Literature survey also reveals RP-HPLC[18] and spectophotometric[19] methods for determination of CEFPO with other drugs. The combined dosage forms of OFLO and CEFPO are available in the market and used as antibacterial drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of OFLO and CEFPO in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or other method for simultaneous estimation of OFLO and CEFPO in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on dual wavelength spectrophotometric method for simultaneous estimation of both drugs in their combined tablet dosage form.

MATERIALS AND METHODS

Apparatus

A shimadzu model 1600 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.10). A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

OFLO and CEFPO bulk powder was supplied by Acme Pharmaceuticals Ltd. Ahmedabad, India. The commercial fixed dose combination product was procured from the local market. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of OFLO (10 mg) and CEFPO (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of OFLO (100 µg/ml) and CEFPO (100 µg/ml).

Method

The working standard solutions of OFLO and CEFPO were prepared separately in methanol having concentration of 10 µg/ml. They were scanned in the wavelength range of 200-400 nm. From the overlain spectra, four wavelengths 224 nm, 247.4 nm, 278.2 nm and 320 nm were selected for quantitation of both the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of OFLO is carried out by measuring the absorbance difference value at between 224 nm and 247.4 nm where CEFPO have same absorbance at both the wavelength. The difference between 224 nm and 247.4 nm is directly proportional to concentration of OFLO in the mixture. The quantitative determination of CEFPO is carried out by measuring the absorbance difference value at 278.2 nm and 320 nm where OFLO have same absorbance at both the wavelength. The difference between 278.2 nm and 320 nm is directly proportional to concentration of CEFPO in the mixture.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[20].

Linearity (Calibration curve)

Appropriate aliquots from the standard stock solutions of OFLO and CEFPO were used to prepare three different sets of dilutions: Series A, B, and C as follows. Series A consisted of different concentration of OFLO (2-12 µg/ml). Aliquot from the stock solution of OFLO (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 2-12 µg/ml. Series B consisted of varying concentrations of CEFPO (4-24 µg/ml). Appropriate volume of the stock solution of CEFPO (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with methanol. Series C comprised of mixture of OFLO and CEFPO having varying concentration of OFLO (2-12 µg/ml) and CEFPO (4-24 µg/ml). The solutions of OFLO and CEFPO were prepared by transferring 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml equivalent to 2, 4, 6, 8, 10, 12 µg/ml from the stock solution of OFLO and 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 ml equivalent to 4, 8, 12, 16, 20, 24 ug/ml CEFPO (100 µg/ml) into a series of 10 ml volumetric flasks and the volume was adjusted up to the mark with methanol. The absorbance of the solutions of series A, B and C were measured at 278.2 nm (λ1), 320 nm (λ2), 224 nm (λ3) and 247.4 nm (λ4). The difference in absorbance between 278.2 nm and 320 nm is due to the CEFPO and was plotted against CEFPO concentration (µg/ml). The difference in absorbance between 224 nm and 247.4 nm is due to the OFLO and was plotted against OFLO concentration (µg/ml) and two different regression equations were obtained.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for OFLO and CEFPO (10 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of OFLO and CEFPO (4, 8, 12 µg/ml for OFLO and 8, 16, 24 µg/ml for CEFPO). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of OFLO and CEFPO by the standard addition method. Known amounts of standard solutions of OFLO and CEFPO were at added at 50, 100 and 150 % level to prequantified sample solutions of OFLO and CEFPO (4 µg/ml for both drug). The amounts of OFLO and CEFPO were estimated by applying obtained values to the respective regression line equations.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[20].

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of OFLO and CEFPO in combined tablet dosage form

Twenty tablets were weighed and the average weight was calculated. The tablet powder equivalent to 10 mg of OFLO and 10 mg of CEFPO were weighed and transferred to 100 ml volumetric flask. Methanol (50 ml) was added and sonicated for 20 min. The volume is adjusted up to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41. The solution was suitably diluted with methanol to get a final concentration of 10 µg/ml of CEFPO and 10 µg/ml of OFLO. The absorbances of final solution were recorded at selected wavelengths for determination of OFLO and CEFPO. The analysis procedure was repeated three times with tablet formulation.

RESULTS AND DISCUSSION

The solution of OFLO and CEFPO were prepared separately in methanol and scanned in the UV range of 200 - 400 nm. From the overlain spectra of both drugs, four specific wavelengths are selected. The absorbance at 278.2 nm (λ1) and 320 nm (λ2) wavelengths was found to be with same absorbance for OFLO. These two selected wavelengths were employed to determine the concentration of CEFPO from the mixture of OFLO and CEFPO. The difference in absorbance at these two wavelengths ($A_{278.2}$– A_{320}) cancels out the contribution of absorbance of OFLO in mixture. Similarly, the absorbance at 224 nm (λ3) and 247.4 nm (λ4) wavelengths was found to be with same absorbance for CEFPO. These two selected wavelengths were employed to determine the concentration of OFLO from the mixture of OFLO and CEFPO. The difference in absorbance at these two wavelengths (A_{224}– $A_{247.4}$) cancels out the contribution of absorbance of CEFPO in mixture.

The proposed method was found to be simple, sensitive, rapid, accurate, precise and economic for the routine simultaneous estimation of two drugs. The linearity range for ofloxacin and cefpodoxime proxetil were found to be 2-14 µg/ml and 4-24 µg/ml respectively. Regression analysis data and summary of all validation parameters is given in Table 1. Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for both the drugs. Accuracy was determined by calculating the recovery and the mean was determined. The LOD and LOQ were found to be 0.332 and 1.09 µg/ml respectively for OFLO and 0.30 and 0.99 µg/ml respectively for CEFPO indicates sensitivity of the proposed method. The method was successfully used to determine the amounts of CEFPO and OFLO present in tablets. The results obtained are in good agreement with the corresponding labeled amount. By observing the validation parameters, the method was found to be sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these drugs in combinations.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of OFLO and CEFPO in tablet dosage form. The method utilizes easily available and cheap solvent for analysis of OFLO and CEFPO hence the method was also economic for estimation of OFLO and CEFPO from tablet dosage form. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of OFLO and CEFPO in method, hence it can be conveniently adopted for routine quality control

analysis of the drugs in combined pharmaceutical formulation.

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TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHOD

Parameters	CEFPO	OFLO
Wavelength (nm)	278.2, 320 nm	224, 247.4 nm
Beer's Law Limit (µg/ml)	4-24	2-12
Regression equation (y = a + bc)	y = 0.0173x - 0.0021	y = 0.0184x - 0.013
Slope (b)	0.0173	0.0184
Intercept (a)	-0.0021	-0.013
Correlation Coefficient (r^2)	0.9994	0.9991
Sandell's sensitivity (mcg/cm^2/0.001 AU)	0.0595	0.0574
Molar extinction co-efficient (L mol^{-1} cm^{-1}/ M^{-1} cm^{-1})	11653.84	7625.54
Accuracy (Recovery) (n=3)	98.81 ± 0.27	99.87 ± 1.18
Repeatability (% RSD[a], n=6)	0.78	1.02
Interday (n=3) (% RSD)	0.58-1.53 %	0.59-1.95 %
Intraday(n=3) (% RSD)	0.37-1.43 %	0.53-1.91 %
LOD[b]	0.30 µg/ml	0.332 µg/ml
LOQ[c]	0.99 µg/ml	1.09 µg/ml
Assay ± SD	99.87 ± 1.18	98.81 ± 0.27

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification

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TABLE 2: RECOVERY DATA OF PROPOSED METHOD

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Recovery ± S. D. (n = 5)
	I	4	50	99.97 ± 1.28
OFLO	II	4	100	99.80 ± 1.50
	III	4	150	99.84 ± 0.77
	I	4	50	99.97 ± 0.29
CEFPO	II	4	100	99.90 ± 0.36
	III	4	150	96.56 ± 0.16

S. D. is standard deviation and n is number of replicate

TABLE 3: ANALYSIS OF OFLO AND CEFPO BY PROPOSED METHOD

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n=6)	
	CEFPO	OFLO	CEFPO	OFLO)	CEFPO	OFLO
Brand I	200	200	200.8	199	100.40	99.50
Brand II	200	200	201.4	198.6	100.70	99.30

S. D. is standard deviation and n is number of replicate

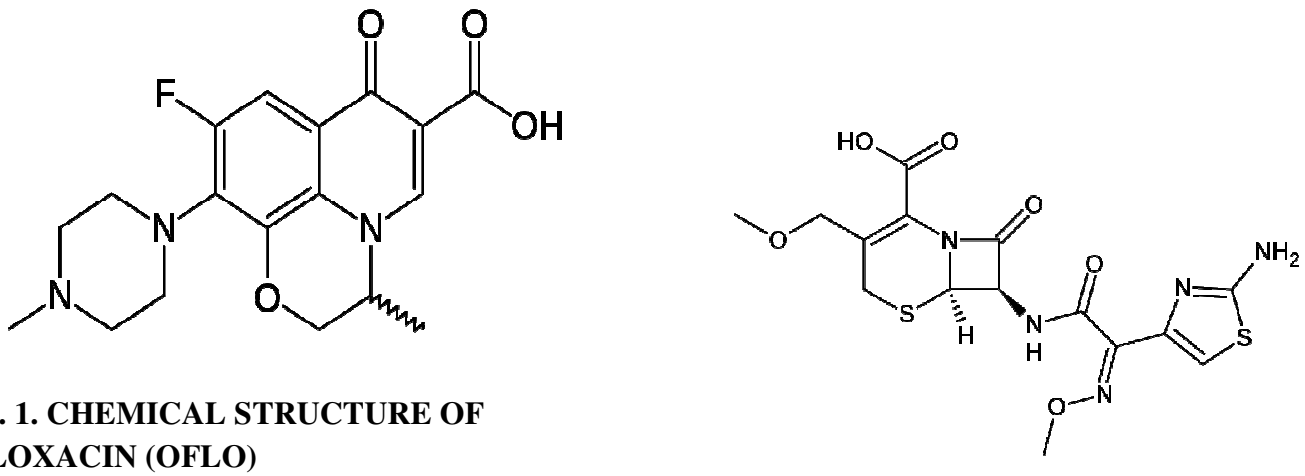

FIG. 1. CHEMICAL STRUCTURE OF OFLOXACIN (OFLO)

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FIG. 2. CHEMICAL STRUCTURE OF CEFPODOXIME PROXETIL (CEFPO)

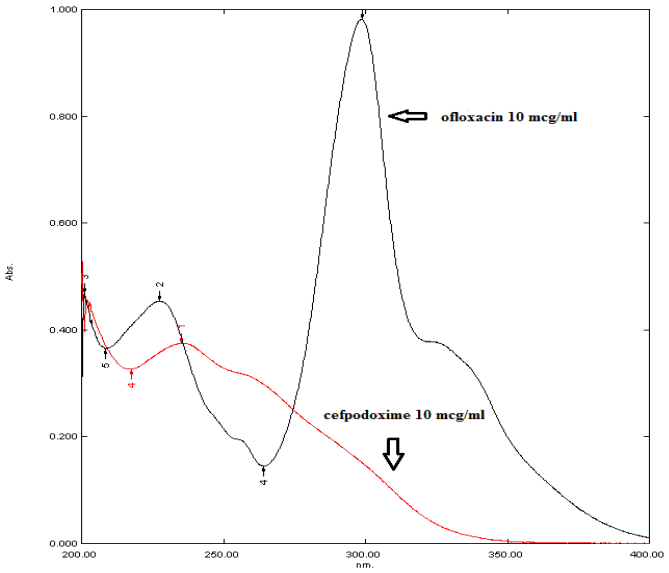

FIG. 3. OVERLAIN ZERO-ORDER ABSORPTION SPECTRA OF OFLO AND CEFPO IN METHANOL

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