IJPSR (2012), Vol. 3, Issue 02 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 October, 2011; received in revised form 19 November, 2011; accepted 22 January, 2012

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF CEFPODOXIME PROXETIL AND OFLOXACIN IN TABLETS

M. N. Shah*, Dr. H. U. Patel and C. N. Patel

Department of Quality Assurance, Shri Sarvajanik Pharmacy College, Nr. Arvind Baug, Mehsana- 384001, Gujarat, India

Keywords:
Cefpodoxime proxetil,
Ofloxacin,
Simultaneous equation method,
Tablets,
Validation

Correspondence to Author:

M. N. Shah

Department of Quality Assurance, Shri Sarvajanik Pharmacy College, Nr. Arvind Baug, Mehsana- 384001, Gujarat, India

ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of cefpodoxime proxetil and ofloxacin in combined tablet dosage form. The method is based on the simultaneous equations for analysis of both the drugs using methanol as solvent. Cefpodoxime proxetil has absorabance maxima at 236 nm and ofloxacin has absorbance maxima at 299 nm in methanol. The linearity was obtained in the concentration range of 5-29 µg/ ml and 1-13 µg/ml for cefpodoxime proxetil and ofloxacin respectively. The concentrations of the drugs were determined by using simultaneous equations at both the wavelengths. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found. The suitability of this method for the quantitative determination of cefpodoxime proxetil and ofloxacin was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of cefpodoxime proxetil and ofloxacin in pharmaceutical dosage form. The result of analysis has been validated statistically and by recovery studies.

INTRODUCTION: Cefpodoxime proxetil (CPD) (Figure 1) is chemically, 1-(isopropoxy carbonyloxy) ethyl (6R, 7R)-7-[2- (2-amino- 4- thiazolyl)- (z)- 2- (methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate [1], is a third generation cephalosporin antibiotic. It is used for infections of the respiratory tract, urinary tract and skin and soft tissues [2]. Cefpodoxime proxetil is official in IP and USP. IP [3] and USP [4] describe liquid chromatography method for its estimation. Literature survey reveals HPTLC [5] method for the determination of CPD. Literature survey also reveals RPHPLC [6] and spectrophotometric [7] methods for determination of CPD with other drugs. Ofloxacin (OFL) (Figure 2) is chemically, 9-fluro-2-3 dihydro-3-methyl-10-(4-methyl 1- piperazinyl) - 7- oxo- 7H- pyrido [1, 2, 3-de] 1, 4benzoxazine-6-carboxylic acid [8], is a fluoroquinolone antibacterial agent used in the treatment of chalmydia or chlamydophila infections including nongonococcal urethritis and in mycobacterial infections such as leprosy [9].

It is official in IP, BP and USP. IP [10], BP [11] and USP [4] describe potentiometric method for its estimation. Literature survey reveals first derivative fluorescence spectroscopy [12], HPLC with fluorescence detector for estimation of ofloxacin in human plasma [13]. Literature survey also reveals spectrophotometric [14], RP-HPLC and HPTLC [15] methods for determination of OFL with other drugs. The combined dosage forms of CPD and OFL are available in the market and used in Urinary

tract infection and Typhoid. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of CPD and OFL in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or chromatographic method for simultaneous estimation of CPD and OFL in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation for estimation of both drugs in their combined tablet dosage forms.

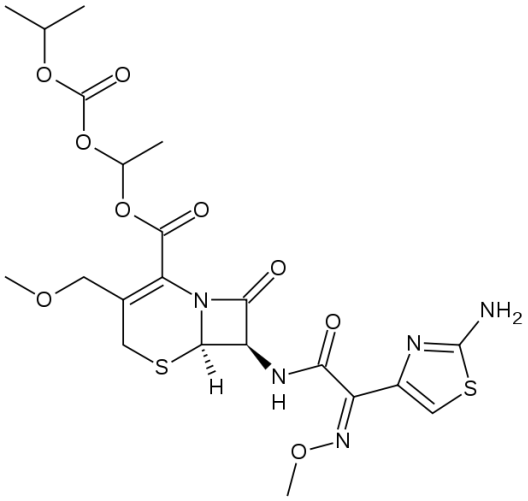

FIG. 1: CHEMICAL STRUCTURE OF CEFPODOXIME PROXETIL (CPD)

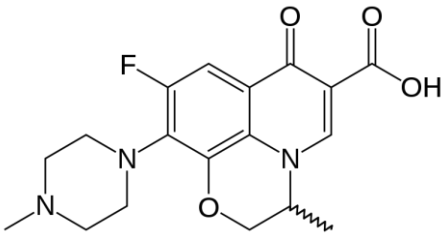

FIG. 2: CHEMICAL STRUCTURE OF OFLOXACIN (OFL)

MATERIALS AND METHODS:

Apparatus: A Shimadzu model 1700(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 2.31). An Electronic analytical balance (Acculab) and an ultrasonic bath were used in the study.

Reagents and Materials: CPD and OFL bulk powder was gifted by Corona Remedies Pvt. Ltd., Ahmadabad, Gujarat, India. The commercial fixed dose combination product was procured from the local market. Methanol AR Grade was procured from S.D.Fine Chemicals Ltd., Mumbai, India.

Preparation of standard stock solution: An accurately weighed quantity of CPD (10 mg) and OFL (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 CPD (100 µg/ml) and OFL (100 µg/ml).

Method: In simultaneous equation method, seven working standard solutions having concentration 5, 9, 13, 17, 21, 25, 29 µg/ml for CPD and 1, 3, 5, 7, 9, 11, 13 µg/ml for OFL were prepared in methanol and the absorbance at 236 nm (λ-max of CPD) and 299 nm (λ-max of OFL) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations

$$C_x = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2} \quad \text{..... (1)}$$

$$C_y = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2} \quad \text{..... (2)}$$

Where A_1, A_2 are absorbance of mixture at 236 nm (λ_1) and 299 nm (λ_2) respectively, ax_1 and ax_2 are absorptivities of CPD at λ_1 and λ_2 respectively, ay_1 and ay_2 are absorptivities of OFL at λ_1 and λ_2 respectively, C_x and C_y are concentrations of CPD and OFL respectively.

Validation of Proposed Method:

Linearity (Calibration curve): The calibration curves were plotted over a concentration range of 5-29 µg/ml for CPD and 1-13 µg/ml OFL. Accurately measured standard stock solutions of each CPD (0.5, 0.9, 1.3, 1.7, 2.1, 2.5 and 2.9 ml) and OFL (0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with methanol. The absorbances of solution were then measured at 236 nm and 299 nm. The calibration curves were constructed by plotting absorbances

versus concentration and the regression equations were calculated.

Precision:

- **Intraday:** Mixed solution containing 3-11 µg/ml of both was analyzed three times on the same day and %R.S.D was calculated.

- **Interday:** Mixed solution containing 3-11 µg/ml of both was analyzed on three different days and %R.S.D was calculated.

Accuracy: Accuracy was determined by calculating recovery of CPD and OFL by the standard addition method. From working sample solution of test (100 µg/ml of both), 0.5 ml of solution were taken and increasing aliquots of combined working standard solution (0.4, 0.5, and 0.6 ml from 100 µg/ml of both) were added and diluted to 10 ml with methanol. These solutions were prepared in triplicate. Absorbance of solution was measured at selected wavelength for CPD and OFL.

The amount of CPD and OFL was calculated at each level by simultaneous equation method and % recoveries were computed.

Limit of detection and Limit of quantitation: The limit of detection (LOD) and the limit of quantitation (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.

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

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

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

Analysis of CPD and OFL in combined tablet: Twenty tablets were weighed and the average weight was calculated. The tablet powder equivalent to 10 mg of CPD and 10 mg of OFL 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 5 µg/ml of CPD and 5 µg/ml of OFL. The absorbances of the sample solution i.e. A1 and A2 were recorded at 236 nm (λ-max of CPD) and 299 nm (λ-max of OFL) respectively, Relative concentration of two drugs in the sample was calculated using above equation (1) and (2).

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

Parameters	CPD	OFL
Wavelength range (nm)	236	299
Beer's law limit (µg/ml)	5-29	1-13
Regression equation (y = mx + c)	y = 0.0312x - 0.0006	y = 0.1024x + 0.0232
Slope	0.0312	0.1024
Intercept	0.0006	0.0232
Correlation Coefficient (r^2)	0.9999	0.9996
System Precision (%R.S.D)[a]		
1. Intraday Precision(n = 3)	0.39-1.10%	0.21-0.80%
2. Interday Precision(n = 3)	0.43-1.78%	0.43-1.26%
Accuracy (% recovery) (n = 3)	99.98-101.35%	98.11-101.44%
LOD[b] (µg/ml)	0.317	0.083
LOQ[c] (µg/ml)	0.961	0.253
Assay (±S.D.)[d] (n = 3)	100.8 ± 0.84	97.6 ± 1.47

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantitation. [d]SD is Standard deviation and n is number of replicates

RESULT AND DISCUSSION: In simultaneous equation method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength [16], which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 236 nm (λ-max of CPD) and 299 nm (λ-max of OFL) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of CPD (236 nm) and OFL (299 nm) in methanol is shown

in **Figure 3**. The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined (**Table 2**). The method was successfully used to determine the amounts of CPD and OFL present in the tablet dosage forms. The results obtained were in good agreement with the corresponding labeled amount (**Table 3**). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs.

TABLE 2: RECOVERY DATA OF PROPOSED METHOD

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount added (%)	% Mean recovery (±S.D.) (n = 3)
CPD	I	5	4	80	100.49 ± 1.74
	II	5	5	100	99.98 ± 1.18
	III	5	6	120	101.35 ± 1.36
OFL	I	5	4	80	98.11 ± 0.43
	II	5	5	100	98.17 ± 0.86
	III	5	6	120	101.44 ± 1.12

S.D. is Standard deviation and n is number of replicates

TABLE 3: ANALYSIS OF CPD AND OFL BY PROPOSED METHOD.

Tablet	Labeled claim (mg)		Amount found (mg)		% Label claim (±S. D.) (n = 3)	
	CPD	OFL	CPD	OFL	CPD	OFL
I	200	200	201.6	195.2	100.8 ± 0.84	97.6 ± 1.47

S.D. is Standard deviation and n is number of replicates

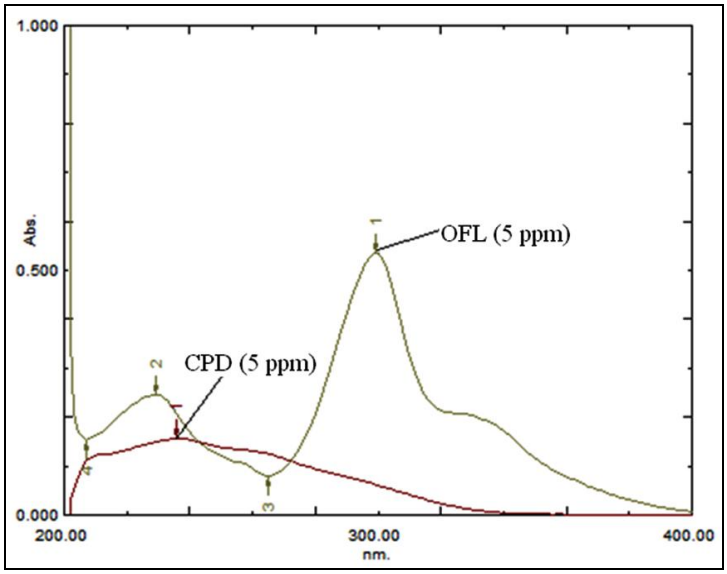

FIG. 3: OVERLAIN ABSORPTION SPECTRA OF CPD (236 NM) AND OFL (299 NM) IN METHANOL

ACKNOWLEDGEMENT: The authors are thankful to Corona Remedies Pvt. Ltd., Ahmedabad, Gujarat, India for providing gift sample of CPD and OFL for research. The authors are highly thankful to Shri Sarvajanik Pharmacy College, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

CONCLUSION: The developed simultaneous equation method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of CPD and OFL. The developed method was validated as par ICH guidelines. Statistical analysis proved that the method is repeatable and selective for the analysis of CPD and OFL in combination as a single drug in bulk as well as in pharmaceutical formulations.

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