JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Development and Validation of First Order Derivative 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 economical first derivative spectrophotometric method for the simultaneous determination of ofloxacin and cefpodoxime proxetil in combined tablet dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra was obtained in methanol and the determinations were made at 236.4 nm (ZCP of cefpodoxime proxetil) for ofloxacin and 208.8 nm (ZCP of ofloxacin) for cefpodoxime proxetil. The linearity was obtained in the concentration range of 2-12 µg/ml for ofloxacin and 4-24 µg/ml for cefpodoxime proxetil. The mean recovery was 99.80 ± 1.50 and 99.90 ± 0.36 for ofloxacin and cefpodoxime proxetil, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of ofloxacin and cefpodoxime proxetil in pharmaceutical tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: Ofloxacin, Cefpodoxime proxetil, First order derivative spectrophotometric method, Tablet, Validation.

Article history:
Received 9 Oct 2011
Accepted 11 Oct 2011
Available online 13 Oct 2011

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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], 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 spectofluorimetric[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 spectofluorimetric[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], is a third generation cephalosporin antibiotic. It is used for infections of the respiratory tract, urinary tract and skin and soft tissues. It has greater activity against staphylococcus aureus[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 spectofluorimetric[19] methods for determination of CEFPO with other drugs. The combined dosage forms of OFLO and CEFPO are available in the market for the prophylaxis and treatment of chronic asthma and chronic bronchitis in pediatrics. 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 and economical spectrophotometric method based on dual wavelength spectrophotometric method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS AND METHODS

Apparatus

A double beam UV/Visible spectrophotometer (shimadzu model 1700, Japan) 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. An analytical balance (Sartorius CP224S, Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

OFLO and CEFPO bulk powder was kindly gifted by Acme Pharmaceuticals Ltd. Ahmedabad, 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 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).

Methodology

The standard solutions of OFLO (10 µg/ml) and CEFPO (10 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 1 nm. The two spectra were overlain and it appeared that OFLO showed zero crossing at 208.8 nm, while CEFPO showed zero crossing at 236.4 nm. At the zero crossing point (ZCP) of OFLO (208.8 nm), CEFPO showed a first-derivative absorbance, whereas at the ZCP of CEFPO (236.4 nm), OFLO showed a first-derivative absorbance. Hence 236.4 and 208.8 nm was selected as analytical wavelengths for determination of OFLO and CEFPO, respectively. These two wavelengths can be employed for the determination of OFLO and CEFPO without any interference from the other drug in their combined dosage formulations.

Validation of the proposed method

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

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-12 µg/ml for OFLO and 4-24 µg/ml for CEFPO. Accurately measured standard solutions of OFLO (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) and CEFPO (0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. First-derivative absorbance (D1) was measured at 236.4 nm for OFLO and 208.8 nm for CEFPO. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solution (n = 6) for OFLO and CEFPO (10 µg/ml) without changing the parameter of the first-derivative 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 recovery of OFLO and CEFPO by the standard addition method. Known amounts of standard solutions of OFLO and CEFPO were added at 50, 100 and 150 % level to prequantified sample solutions of OFLO and CEFPO (4 µg/ml for each drug). The amounts of OFLO and CEFPO were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times.

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[19].

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

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

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

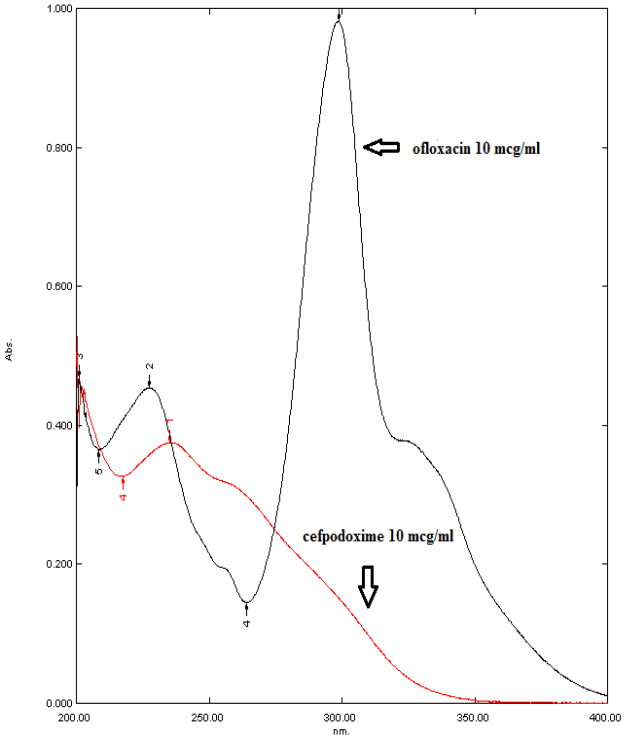

Figure 1: Overlain zero-order absorption spectra of OFLO and CEFPO in methanol

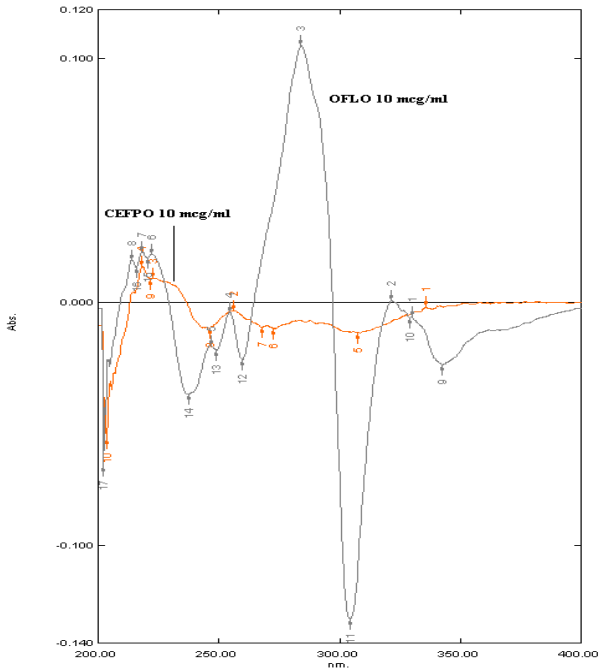

Figure 2: Overlain first-order derivative spectra of OFLO and CEFPO in methanol

Analysis of OFLO and CEFPO in combined tablet dosage form

Twenty Tablets were weighed and powdered. The powder equivalent to 10 mg of OFLO and 10 mg of CEFPO was transferred to a 100 ml volumetric flask. Methanol (50 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. This solution is expected to contain 100 µg/ml of OFLO and 100 µg/ml of CEFPO. This solution (1.0 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of OFLO (10 µg/ml) and CEFPO (10 µg/ml). The responses of the sample solution were measured at 236.4 nm and 208.8 nm for quantification of OFLO and CEFPO, respectively. The amounts of the OFLO and CEFPO present in the sample solution were calculated by fitting the responses into the regression equation for OFLO and CEFPO in the proposed method.

RESULTS AND DISCUSSION

The standard solutions of OFLO and CEFPO were scanned separately in the UV range, and zero-order spectra (Figure 1) thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 1 nm. The two derivative spectra showed maximum absorbance at 236.4 nm (ZCP of CEFPO) for OFLO and 208.8 nm (ZCP of OFLO) for CEFPO. First-derivative absorbances (D1) were recorded 236.4 nm for OFLO and 208.8 nm for CEFPO (Figure 2). First derivative spectra give good quantitative determination of both the drugs at their respective without any interference from the other drug in their combined dosage formulations.

Linear correlation was obtained between absorbance and concentration of OFLO and CEFPO in the concentration ranges of 2-12 µg/ml and 4-24 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 1). The RSD values for OFLO and CEFPO were found to be 0.46 and 0.57 %, respectively (Table 1). The low values of relative standard deviation (less than 2 %) indicate that the proposed method is repeatable. The low RSD values of interday (0.34-0.67 and 0.48-0.73 %) and intraday (0.35-1.07 and 0.52-1.22 %) for OFLO and CEFPO, respectively, reveal that the proposed method is precise (Table 1). LOD values for OFLO and CEFPO were found to be 0.140 and 0.164 µg/ml, respectively and LOQ values for OFLO and CEFPO were found to be 0.424 and 0.498 µg/ml, respectively (Table 1). These data show that proposed method is sensitive for the determination of OFLO and CEFPO.

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.80 ± 1.50 and 99.90 ± 0.36 % for OFLO and CEFPO, respectively (Table 2). The results of recovery studies indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine OFLO and CEFPO in their combined dosage form. The results obtained for OFLO and CEFPO were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of OFLO and CEFPO in pharmaceutical dosage forms.

Table 1: Regression analysis data and summary of validation parameters for the proposed method

PARAMETERS	First-derivative UV Spectrophotometry	
	OFLO at 236.4 nm	CEFPO at 208.8 nm
Concentration range (µg/ml)	2-12	4-24
Regression equation (y = a + bc)	y = 0.0032x - 0.0007	y = 0.0011x + 0.0084
Slope (b)	0.0032	0.0011
Intercept (a)	-0.0007	0.0084
Correlation Coefficient (r^2)	0.9993	0.9990
Sandell's sensitivity (µg/cm^2/0.001 A.U.)	0.0333	0.0254
Accuracy (% recovery)(n = 6)	99.80 ± 1.50	99.90 ± 0.36
Repeatability (%RSD[a], n = 6),	0.46	0.57
Interday (n = 3) (%RSD)	0.34-0.67 %	0.48-0.73 %
Intraday(n = 3) (%RSD)	0.35-1.07 %	0.52-1.22 %
LOD[b] (µg/ml)	0.140 µg/ml	0.164 µg/ml
LOQ[c] (µg/ml)	0.424 µg/ml	0.498 µg/ml

Table 2: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	% Mean recovery ± S.D. (n = 6)
OFLO	I	4	2	99.51 ± 0.75
	II	4	4	100.5± 1.39
	III	4	6	100.4 ± 1.62
CEFPO	I	4	2	99.42 ± 0.91
	II	4	4	99.71 ± 1.79
	III	4	6	99.98 ± 1.45

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

Table 3: Analysis of cefpo and oflo by proposed method

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

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

CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 2-12 µg/ml and 4-24 µg/ml for OFLO and CEFPO, respectively with co-efficient of correlation, (r^2)=0.9993 and (r^2) = 0.9990 for OFLO and CEFPO, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of OFLO and CEFPO. The method can be used for the routine analysis of the OFLO and CEFPO in combined dosage form without any interference of excipients.

ACKNOWLEDGEMENT

The authors are thankful to Acme Pharmaceuticals Ltd., Ahmedabad, Gujarat, India for providing gift sample of OFLO and CEFPO for research. The authors are highly thankful to Center for Health Science Studies, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

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