Journal of Applied Pharmaceutical Science
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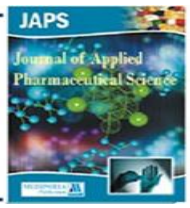

ISSN: 2231-3354
Received on: 16-09-2011
Revised on: 19-07-2011
Accepted on: 22-08-2011

Simultaneous spectrophotometric determination of cefpodoxime proxetil and ofloxacin in tablets

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of cefpodoxime proxetil and ofloxacin in combined tablet dosage form. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. Cefpodoxime proxetil and ofloxacin show an isoabsorptive point at 273.2 nm in methanol. The second wavelength used is 297 nm, which is the λ-max of ofloxacin in methanol. The linearity was obtained in the concentration range of 2-14 µg/ml for both cefpodoxime proxetil and ofloxacin. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ-max of ofloxacin. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found. The results of analysis have been validated statistically and by recovery studies.

Key words Cefpodoxime proxetil, ofloxacin, absorbance ratio method, isoabsorptive point, validation, simultaneous.

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INTRODUCTION

Ofloxacin (OFLO) (Figure 1) 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 (Maryadele et al., 2006), is a fluoroquinolone antibacterial agent used in the treatment of chalmydia or chlamydophila infections including nongonococcal urethritis and in mycobacterial infections such as leprosy. (Sweetman et al., 2007). It is official in IP, BP and USP. IP (Indian Pharmacopoeia., 2010), BP (British Pharmacopoeia., 2010) and USP (United States Pharmacopoeia., 2005) describe potentiometric method for its estimation. Literature survey reveals 1st derivative fluorescence spectroscopy (Juan et al., 2000), solid-phase spectrofluorimetry (Ballestros et al., 2002), HPLC with fluorescence detector for estimation of ofloxacin in human plasma (Wongsinsup et al., 2009), HPLC with fluorescence detection for determination of ofloxacin in human aqueous humor (Basci et al., 1997) and chemiluminescence (Francis et al., 2005) methods for determination of OFLO in pharmaceutical dosage forms as well as in biological fluids. Literature survey also reveals spectrophotometric (Patel et al., 2007), RP-HPLC and HPTLC (Puranik et al., 2010) methods for determination of OFLO with other drugs. Cefpodoxime proxetil (CEFPO) (Figure 2) is chemically, 1-(isopropoxy carbonyloxy) ethyl(6R,7R)-7-[2-(2-amino-4-thiazolyl)-(z)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate (Maryadele et al., 2006), is a third generation cephalosporin antibiotic. It is used for infections of the respiratory tract, urinary tract and skin and soft tissues (Sweetman et al., 2007). Cefpodoxime proxetil is official in IP and USP. IP (Indian Pharmacopoeia., 2010) and USP (United States Pharmacopoeia., 2005)

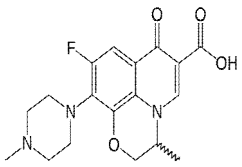

Fig. 1: Chemical structure of ofloxacin

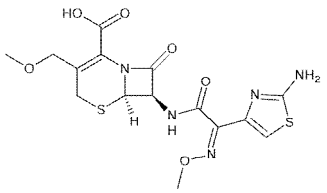

Fig. 2: Chemical structure of cefpodoxime proxetil (CEFPO)

describe liquid chromatography method for its estimation. Literature survey reveals HPTLC (Darji et al., 2007) method for the determination of CEFPO. Literature survey also reveals RP-HPLC (Singh et al., 2010) and spectrophotometric (Gandhi et al., 2009) 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 chromatographic method for simultaneous estimation of OFLO and CEFPO in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method based on absorbance ratio method (Q-analysis) for simultaneous estimation of both drugs in their combined tablet dosage forms.

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 2.10). A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

CEFPO and OFLO bulk powder was 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 CEFPO (10 mg) and OFLO (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 CEFPO (100 µg/ml) and OFLO (100 µg/ml).

Method

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that CEFPO and OFLO show an isoabsorptive point at 273.2 nm. The second wavelength used is 297 nm, which is the λ-max of OFLO. Seven working standard solutions having concentration 2, 4, 6, 8, 10, 12 and 14 µg/ml for CEFPO and 2, 4, 6, 8, 10, 12 and 14 µg/ml for OFLO were prepared in methanol and the absorbances at 273.2 nm (isoabsorptive point) and 297 nm (λ-max of OFLO) were measured and absorptivity coefficients were calculated using calibration curve.

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

$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1/aX_1 \quad \text{.....(3)}$$

$$C_Y = (A_1/aX_1) - C_X \quad \text{.....(4)}$$

Where, A_1 and A_2 are absorbances of mixture at 273.2 nm and 297 nm; aX_1 and aY_1 are absorptivities of CEFPO and OFLO at 273.2 nm; aX_2 and aY_2 are absorptivities of CEFPO and OFLO respectively at 297 nm; $Q_M = A_2 / A_1$, $Q_X = aX_2 / aX_1$ and $Q_Y = aY_2 / aY_1$.

Validation of the proposed method

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-14 µg/ml for each CEFPO and OFLO. Accurately measured standard stock solutions of each CEFPO and OFLO (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml) 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 273.2 nm and 297 nm. The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated.

Method precision (repeatability)

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

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of CEFPO and OFLO (4, 8 and 12 µg/ml).

Accuracy (recovery study)

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

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

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

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

Analysis of CEFPO and OFLO in combined tablet

Twenty tablets were weighed and the average weight was calculated. The tablet powder equivalent to 10 mg of CEFPO and 10 mg of OFLO 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 the sample solution i.e. A_1 and A_2 were recorded at 273.2 nm (isoabsorptive point) and 297 nm (λ-max of OFLO) respectively, and ratios of absorbance were calculated, i.e. A_2/A_1. Relative concentration of two drugs in the sample was calculated using above equation (3) and (4). The analysis procedure was repeated three times with tablet formulation.

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

PARAMETERS		CEFPO	OFLO	CEFPO & OFLO
Wavelength range (nm)		297	297	273.2
Beer's law limit (µg/ml)		2 - 14	2 - 14	2 - 14
Regression equation (y = a + bc)		y = 0.0144x + 0.015	y = 0.0969x + 0.039	y = 0.0245x + 0.0055
Slope (b)		0.0144	0.0969	0.0245
Intercept (a)		0.015	0.039	0.0055
Correlation Coefficient (r^2)		0.9987	0.9992	0.9989
Sandell's sensitivity (µg/cm^2/0.001 A.U.)		0.062	0.01	0.039
Molar extinction co-efficient (l mol^{-1} cm^{-1})		8977.36	36140	13995.76 (CEFPO) 9071.14 (OFLO)
Accuracy (Recovery) (n = 3)	Level I	100.5 ± 1.36	101.2 ± 1.12	-
	Level II	99.60 ± 1.18	99.70 ± 0.86	-
	Level III	100.6 ± 1.74	99.44 ± 0.43	-
Method precision (Repeatability) (% RSD, n = 6),		0.95	0.59	0.64
Interday (n = 3) (% RSD[a])		0.32 - 1.37	0.27 - 1.09	0.48 - 1.84
Intraday(n = 3) (% RSD)		0.30 – 1.25	0.24 – 0.99	0.55 - 1.88
LOD[b] (µg/ml)		0.42	0.46	0.36
LOQ[c] (µg/ml)		1.40	1.53	1.20
Assay ± S. D[d]. (n = 3)		100.4 ± 1.04	99.14 ± 1.22	-

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

RESULTS AND DISCUSSION

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength (Beckett et al., 1997), which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 273.2 nm (isoabsorptive point) and 297 nm (λ-max of OFLO) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of CEFPO (236.2 nm) and OFLO (297 nm) showing isoabsorptive point (273.2 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 1). The method was successfully used to determine the amounts of CEFPO and OFLO present in the tablet dosage forms. The results obtained were in good agreement with the corresponding labeled amount (Table 2). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is given in Table 3. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in combined dosage form.

Table 2: Recovery data of proposed method.

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
OFLO	I	4	50	101.2 ± 1.12
	II	4	100	99.70 ± 0.86
	III	4	150	99.44 ± 0.43
CEFPO	I	4	50	100.5 ± 1.36
	II	4	100	99.60 ± 1.18
	III	4	150	100.6 ± 1.74

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 = 3)	
	CEFPO	OFLO	CEFPO	OFLO	CEFPO	OFLO
I	200	200	199.4	197.9	99.68 ± 0.84	98.95 ± 1.47
II	200	200	202.4	198.6	101.2 ± 1.25	99.32 ± 0.97

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

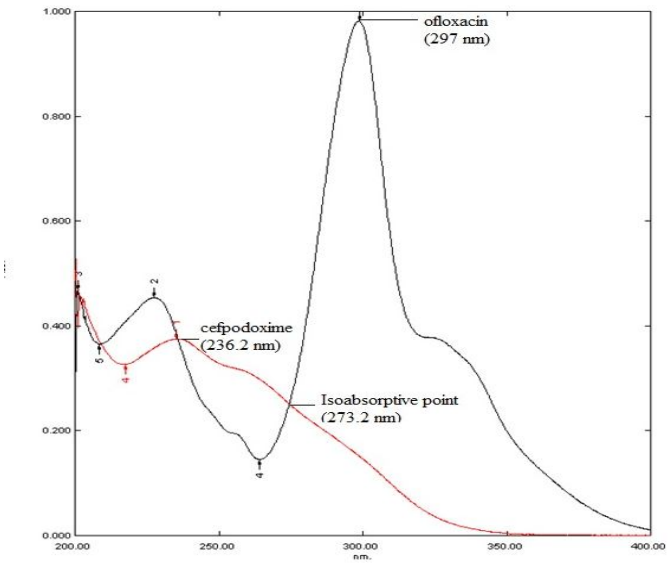

Fig. 3: Overlain absorption spectra of CEFPO (236.2 nm) and OFLO (297 nm) showing isoabsorptive point (273.2 nm) in methanol

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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