INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF CEFIXIME TRIHYDRATE AND OFLOXACIN IN TABLETS

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Article Received on: 06/06/11 Revised on: 10/07/11 Approved for publication: 17/08/11

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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 cefixime trihydrate 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. Cefixime trihydrate and ofloxacin show an isoabsorptive point at 280.2 nm in methanol. The second wavelength used is 291.4 nm, which is the λ-max of cefixime trihydrate in methanol. The linearity was obtained in the concentration range of 2-14 µg/ml for both cefixime trihydrate and ofloxacin. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ-max of cefixime trihydrate. 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.
KEYWORDS: Cefixime trihydrate, Ofloxacin, Absorbance ratio method, Spectrophotometric, Tablet, Validation

INTRODUCTION

Cefixime trihydrate (CEFI), [6R, 7R] – 7- [[(2Z)- 2- (2- amino thiazole- 4-yl)- [(carboxy methoxy) imino] aetyl] amino]-3-ethenyl -8-oxo 5-thia 1-aza bicyclo [4.2.0] oct- 2- ene-2 carboxylic trihydrate (Figure 1), is a third generation orally acting cephalosporin antibiotic[1]. Ofloxacin (OFLO), 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 (Figure 2), is a floroquinolone antibiotic[2]. This combination is used in the treatment of typhoid fever, urinary tract infection, respiratory tract infection, nosocomial infections, soft tissue infections, surgical prophylaxis and intra-abdominal infections[3]. Literature survey reveals spectrophotometric[4], HPLC[5] and HPTLC[6] methods for determination of CEFI in pharmaceutical dosage forms as well as in biological fluids. Literature survey reveals spectofluorimetric[7-8] and HPLC[9-10] methods for determination of OFLO in pharmaceutical dosage forms as well as in biological fluids. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of CEFI and OFLO in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or other method for simultaneous estimation of CEFI and OFLO in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate and economical spectrophotometric method for simultaneous estimation of both drugs in their combined tablet dosage forms.

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

Reagents and Materials

CEFI and OFLO 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 CEFI (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 CEFI (100 µg/ml) and OGLO (100 µg/ml).

Methodology

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 CEFI and OFLO show an isoabsorptive point at 280.2 nm. The second wavelength used is 291.4 nm, which is the λ-max of CEFI. Seven working standard solutions having concentration 2, 4, 6, 8, 10, 12 and 14 µg/ml for CEFI and 2, 4, 6, 8, 10, 12 and 14 µg/ml for OFLO were prepared in methanol, and the absorbances at 280.2 nm (isoabsorptive point) and 291.4 nm (λ-max of CEFI) 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 280.2 nm and 291.4 nm; and aX_1 and aY_1 are absorptivities of CEFI and OFLO at 280.2 nm; aX_2 and aY_2 are absorptivities of CEFI and OFLO respectively at 291.4 nm; and $Q_M = A_2 / A_1$, $Q_X = aX_2 / aX_1$ 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 CEFI and OFLO. Accurately measured standard stock solutions of each CEFI 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 280.2 nm and 291.4 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 CEFI 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 CEFI and OFLO (4, 8 and 12 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of CEFI and OFLO by the standard addition method. Known amounts of standard solutions of CEFI and OFLO were at added at 50, 100 and 150 % level to prequantified sample solutions of CEFI and OFLO (5 µg/ml for both drug). The amounts of CEFI 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[11]

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

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

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

Analysis of CEFI and OFLO in combined tablet dosage form

Twenty tablets were weighed and the average weight was calculated. The tablet powder equivalent to 10 mg of CEFI 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 CEFI and 10 µg/ml of OFLO. The absorbances of the sample solution i.e. A_1 and A_2 were recorded at 280.2 nm (isoabsorptive point) and 291.4 nm (λ-max of CEFI) 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.

RESULTS & 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[12], which was fulfilled in case of both these

drugs. The two wavelengths were used for the analysis of the drugs were 280.2 nm (isoabsorptive point) and 291.4 nm (λ-max of CEFI) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of CEFI (291.4 nm) and OFLO (298 nm) showing isoabsorptive point (280.2 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 CEFI 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.

ACKNOWLEDGEMENT

The authors are thankful to Acme Pharmaceutical Ltd., Ahmedabad, India for providing gift sample of CEFI and OFLO for research. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

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TABLE 1: RECOVERY DATA OF PROPOSED METHOD

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Mean recovery ± S.D. (n = 3)
OFLO	I	5	2.5	7.59	101.2 ± 1.12
	II	5	5	9.97	99.70 ± 0.86
	III	5	7.5	12.43	99.44 ± 0.43
CEFI	I	5	2.5	7.54	100.5 ± 1.36
	II	5	5	9.96	99.60 ± 1.18
	III	5	7.5	12.57	100.6 ± 1.74

TABLE 2: ANALYSIS OF OFLO AND CEFI BY PROPOSED METHOD

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 3)	
	CEFI	OFLO	CEFI	OFLO)	CEFI	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

TABLE 3: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHOD

PARAMETERS		CEFI	OFLO	CEFI & OFLO
Wavelength range (nm)		291.4	291.4	280.2
Beer's law limit (µg/ml)		2 - 14 1	2 - 14	2 - 14
Regression equation (y = a + bc) Slope (b) Intercept (a)		y = 0.043x-0.004 0.043 0.004	y = 0.086x+0.019 0.086 0.019	y = 0.037x+0.008 0.037 0.008
Correlation Coefficient (r^2)		0.9982	0.9990	0.9980
Sandell's sensitivity (µg/cm^2/0.001 A.U.)		0.0240	0.0112	0.0255
Molar extinction co-efficient ($l\ mol^{-1}\ cm^{-1}$)		21328.58	32537.30	19880.3 (CEFI) 14155.9 (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. (n = 3)		100.44 ± 1.04	99.14 ± 1.22	-

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

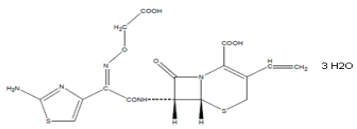

Figure 1: Chemical structure of cefixime trihydrate (CEFI)

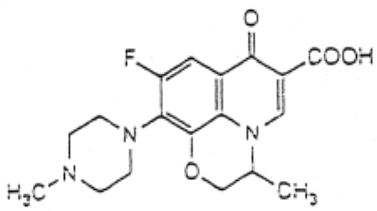

Figure 2: Chemical structure of ofloxacin (OFLO)

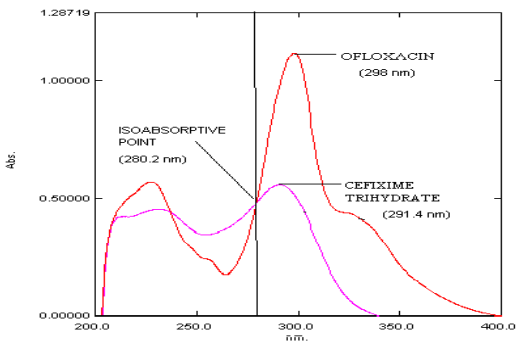

Figure 3: Overlain absorption spectra of CEFI (291.4 nm) and OFLO (298 nm) showing isoabsorptive point (280.2 nm) in methanol

Source of support: Nil, Conflict of interest: None Declared