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Research Article

DEVELOPMENT AND VALIDATION OF DUAL WAVELENGTH SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND OFLOXACIN IN TABLET DOSAGE FORM

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ABSTRACT
The present manuscript describe simple, sensitive, rapid, accurate, precise and economic dual wavelength spectrophotometric method was developed for the simultaneous determination of cefixime trihydrate (CEFI) and ofloxacin (OFLO) 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 350 nm using its absorptivity value and cefixime at 264 nm after deduction of absorbance due to ofloxacin. The two drugs follow Beer-Lanbert's law over the concentration range of 2-14 µg/ml. 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, Dual wavelength spectrophotometric method, 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 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 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 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. 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 Lts., 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).

Development of the methods

The solution of CEFI and OFLO 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 two wavelengths 350 nm and 264 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 value at 350 nm, where CEFI does not have any absorbance and the quantitative determination of CEFI is carried out by subtracting absorbance due to OFLO at 264 nm and the difference between 264 nm and 350 nm is directly proportional to concentration of CEFI in the mixture.

Validation of the proposed method

Linearity (Calibration curve)

Appropriate aliquots from the stock solutions of OFLO and CEFI were used to prepare three different sets of dilutions: Series A, B, and C as follows. Series A consisted of different concentration of OFLO (2-14 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 2-14 µg/ml. Series B consisted of varying concentrations of CEFI (2-14 µg/ml). Appropriate volume of the stock solution of CEFI (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 CEFI having varying concentration of OFLO and CEFI (2-14 µg/ml). The solutions of OFLO and CEFI were prepared by transferring 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml equivalent to 2, 4, 6, 8, 10, 12, 14 µg/ml from the stock solution of OFLO and CEFI (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 264 (λ2) and 350 nm (λ1). The difference in absorbance between 264 nm and 350 nm is due to the CEFI and was plotted against CEFI concentration (µg/ml). The absorbance at 350 nm is due to OFLO only 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 the absorbances of solutions (n=6) of CEFI and OFLO (10 µg/ml for both drugs) without changing the parameters.

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 OGLO (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 10mg 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 final solution were recorded at selected wavelengths for determination of OFLO and CEFI. The analysis procedure was repeated three times with tablet formulation.

RESULTS & DISCUSSION

In this method two specific wavelengths are selected, First wavelength λ1 at which minimum absorbance of OFLO was observed and there was no interference of

CEFI at this wavelength (350 nm). Second wavelength λ2 was the wavelength at which the absorbance of OFLO was same as at λ1, and CEFI was also having some absorbance at this wavelength (264 nm). The absorbance at these two wavelengths was found to be equal for OFLO. These two selected wavelengths were employed to determine the concentration of CEFI from the mixture of OFLO and CEFI. The difference in absorbance at these two wavelengths (A_{264}– A_{350}) cancels out the contribution of absorbance of OFLO 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 ranges for both drugs were found to be 2-14 µg/ml. Precision was calculated as repeatability (relative standard deviation) and intra and inter day variation (% RSD) for both the drugs. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1). The LOD and LOQ were found to be 0.332 and 1.00 µg/ml, respectively for OFLO and 0.420 and 1.27 µg/ml, respectively for CEFI, indicates sensitivity of the proposed method. The method was successfully used to determine the amounts of CEFI and OFLO present in tablets. The results obtained are in good agreement with the corresponding labelled amount (Table 2). Regression analysis data and summary of all the validated parameters is summarised in Table 3. 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.

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REFERENCES

1. Maryadele. J. O' Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station: 2006. p. 1924.
2. Maryadele. J. O' Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station: 2006. p. 6765.
3. Zhao G, Miller MJ, Franzblau S, Wan B, Cephalosporin and quinolone combination: follows synergism. Bio org Med Chem Lett 2010; 5534-7.
4. Virypaxappa BS, Shivaprasad KH, Latha MS. A simple method for the spectrophotometric determination of cefixime in pharmaceuticals. Int J Chemical Eng Research 2010;2:23-30.
5. Andrew J. Falkowski, Zee M. Look, Hideyo Nouguchi, B. Michael Silber. Determination of cefixime in biological samples by RP-HPLC. J Chromatogr 1987;422:145-52.
6. Eric-Jovanovi S, Agbaba D, Zivanov-Stakic D, Vladimirov S. HPTLC determination of cephalosporins in dosage forms. J Pharm Biomed Anal 1998;18:893-98.
7. Juan Gong O, Lii Qiao J, Min Du, Chaun Dong L. Recognization and simultaneous determination of ofloxacin enantiomers by synchronization - 1st derivative fluorescence spectroscopy. Talanta 2000;53:359-65.
8. Ballestros O, LuisVilchez J, Navalon A. Determination of the antibacterial ofloxacin in human urine and serum samples by solid-phase spectrofluorimetry. J Pharm Biomed Anal 2002;30:1103-10.
9. Wongsinsup C, Taesotikul W, Kaewvichit S, Sangsrijan S. Determination of ofloxacin in human plasma by HPLC with fluorescence detector. J Nat Sci 2009;8:165-74.
10. Basci N, Hanioglu K, Soysal H. Determination of ofloxacin in human aqueous humor by HPLC with flourescence detection. J Pharm Biomed Anal 1997;15:663-66.
11. The International conference on harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology:2005.

Table 1: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery ± S.D. (n=3)
OFLO	1	5	2.5	7.49	99.97 ± 1.28
	2	5	5	9.98	99.80 ± 1.50
	3	5	7.5	12.48	99.84 ± 0.77
CEFI	1	5	2.5	7.49	99.97 ± 0.29
	2	5	5	9.99	99.90 ± 0.36
	3	5	7.5	12.07	96.56 ± 0.16

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	200.8	199	100.40	99.50
II	200	200	201.4	198.6	100.70	99.30

Table 3: Regression analysis data and summary of validation parameters for the proposed method

Parameters	CEFI	OFLO
Wavelength (nm)	264, 350 nm	350 nm
Beer's Law Limit (µg/ml)	2-14	2-14
Regression equation (y = a + bc) Slope (b) Intercept (a)	y = 0.022x - 0.0019 0.022 0.0019	y = 0.020x + 0.0023 0.020 0.0023
Correlation Coefficient (r^2)	0.9990	0.9998
Sandell's sensitivity (mcg/cm^2/0.001 AU)	0.0457	0.0473
Molar extinction co-efficient (L mol^{-1} cm^{-1}/ M^{-1} cm^{-1})	11103.08	7638.29
Accuracy (Recovery) (n=3)	98.81 ± 0.27	99.87 ± 1.18
Repeatability (% RSD[a], n=6)	0.73	1.02
Interday (n=3) (% RSD)	0.55-1.56 %	0.60-1.94 %
Intraday(n=3) (% RSD)	0.35-1.47 %	0.52-1.92 %
LOD[b]	0.420 µg/ml	0.332 µg/ml
LOQ[c]	1.270 µg/ml	1.00 µg/ml
Assay ± SD	99.97±0.79	99.95±0.55

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

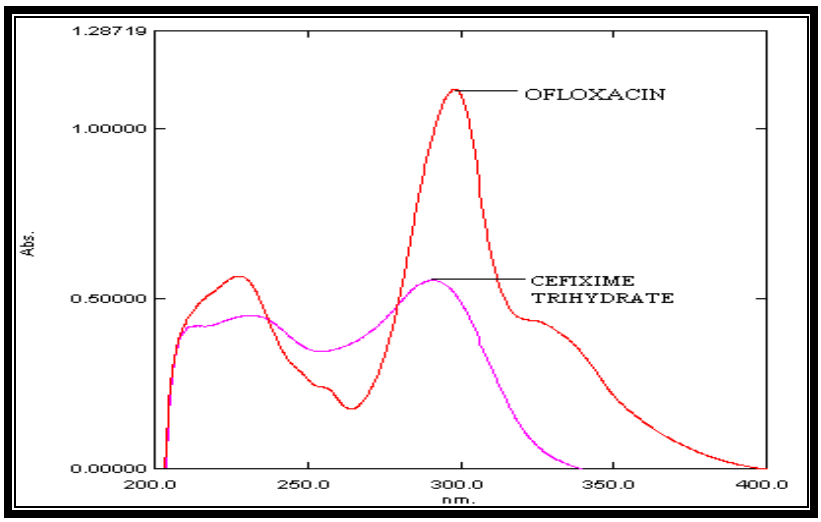

Figure 1: Overlain spectra of CEFI and OFLO in methanol.

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