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Spectrophotometric Simultaneous Determination of Cefixime and Ofloxacin in Combined Tablet Dosage Form by Ratio Derivative and Area Under Curve Method And It's Application To Dissolution Study

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ABSTRACT

A simple, precise and accurate methods for simultaneous determination of Cefixime (CEF) and Ofloxacin (OFL) in combined tablet dosage form has been developed . The method is based on Ratio spectra derivative and Area under curve (AUC) spectrophotometry using methanol and 0.1 N HCl, respectively as solvents. The amplitudes at 319.11 nm and 347.40 nm in the first derivative of the ratio spectra were selected to determine CEF and OFL, respectively and wavelength ranges of 277-279 nm and 296-298 nm were selected to determine CEF and OFL by AUC method in combined formulation. Beer's law is obeyed in the concentration range of 5-25 µg/mL and 4-20 µg/mL by Ratio spectra derivative and Area under curve method, respectively for both the analytes. The % assay in commercial formulation was found to be in the range 99.01 – 100.90 % for CEF and 98.91 – 101.72 % for OFL by the proposed methods. The methods were validated with respect to linearity, precision and accuracy. Recovery was found in the range of 98.60 – 101.80 % for CEF and 98.75 – 100.2% for OFL by ratio derivative method and 98.16 – 100.4% for CEF and 98.89-100.21% for OFL by AUC method respectively for both the Formulations. The methods developed are simple, economical, precise and accurate and can be used for routine quality control of combined tablets. AUC method was successfully applied to carry out dissolution study of commercial tablet formulation by using USP II dissolution test apparatus.

Key words: Cefixime, Ofloxacin, Ratio Spectra Derivative Spectrophotometry, Area Under Curve, Tablet Dissolution study.

INTRODUCTION

Cefixime (CEF) is an oral third generation cephalosporin antibiotic. chemically it is (6R,7R)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(carboxymethoxyimino)acetyl]amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2- carboxylic acid, clinically used in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such

as bronchitis, and urinary-tract infections [1]. Literature survey reveals that cefixime can be estimated spectrophotometrically [2-3]. HPLC [4-5] and by HPTLC [6] individually or with other drugs in bulk drugs and in human plasma, Ofloxacin (OFL), is an antimicrobial drug and chemically it is 9-fluro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperizinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxaine-6-carboxylic acid, It is mainly used for the treatment of urinary tract infection and sexually transmitted diseases[1]. Extensive literature survey reveals that various analytical methods have been reported for the estimation of OFL in single and in combination dosage form such as Spectrophotometric [7-10], Potentiometry and Conductometry [11], HPLC [12-14] and LC/MS/MS[15]. As per our knowledge there is no spectroscopic method available in the literature for the simultaneous estimation of CEF and OFL in combined dosage form. Therefore aim of the study was to develop and validate Ratio Derivative and AUC spectroscopic methods for the determination of CEF and OFL in tablet dosage form and application of AUC method for dissolution study. The proposed methods were validated as per the International Conference on Harmonization (ICH) guidelines [16].

MATERIALS AND METHODS

Instrumentation

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells was used. Electronic balance (Model Shimadzu AUW-220D) was used for weighing. Electro lab, Tablet dissolution tester (Model TDT-08L) USP II was used for dissolution study.

Reagents and chemicals

Pure drug sample of CEF, % purity 99.86 and OFL, % purity 99.92 was kindly supplied as a gift sample by Alkem Pharmaceutical Pvt. Ltd. Mumbai and Mapro Pharmaceutical Pvt Ltd., wadhwan, Gujarat, respectively. These samples were used without further purification. Spectroscopy grade methanol and analytical reagent grade HCl was used throughout the study. Tablets each containing 200 mg of CEF and OFL used for analysis were MAHACEF-PLUS(Formulation - TI) and OMNICEF-O 200(Formulation - TII) manufactured by Akums Drugs And Pharmaceutical Ltd. Haridwar and Otsira Genetica and Pharmaceutical Ltd. India, respectively.

Preparation of Standard Stock Solutions and calibration Curve

Standard stock solutions of pure drug containing 1000 µg/mL of CEF and OFL were prepared separately in methanol and 0.1 N HCl for method A and B, respectively. Standard stock solutions were further diluted with methanol for method A and with distilled water for method B to get working standard solutions of analytes in the concentration range of 5-25 µg/mL and 4-20 µg/mL for Ratio spectra derivative and Area under curve method, respectively . First derivative amplitudes (at interval 1.2 and filter size 9) of ratio spectra were measured at 319.11 nm and 347.40 nm for CEF and OFL, respectively. First derivative amplitudes of ratio spectra and concentrations were used to construct calibration curves for method A. Integrated area under curve was obtained between wavelength ranges of 277-279 nm and 296-298 nm for CEF and OFL, respectively for AUC method. Integrated area under curve was used to construct two simultaneous equations and these equations were solved and used (3 and 4) to calculate amount of analytes in sample solutions.

Preparation of Sample Stock Solution and Formulation analysis

For method A, twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 100 mg of CEF (100 mg of OFL) was weighed and dissolved in the 80 mL of methanol (0.1 N HCl for method B) with the aid of ultrasonication for 15 min and solution was filtered through Whatman paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with same solvent, adding washings to the volumetric flask and volume was made up to the mark with the solvent. The solution was suitably diluted further with methanol (with distilled water for method B) to get required final concentration of CEF (100µg/mL) and OFL (100 µg/ mL).

Theoretical aspects

Method A: Ratio Derivative

The method involves dividing the spectrum of mixture by the standardized spectra of each of the analyte to get ratio spectra and first derivative of ratio spectrum was obtained which was independent of concentration of divisor (Fig. 1). Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. The ratio spectra of different CEF standards at increasing concentrations were obtained by dividing each with the stored spectrum of the standard solution of OFL (15 µg/mL) as shown in (Fig 1A).Wavelength 319.11 nm was selected for the quantification of CEF in CEF + OFL mixture. The ratio and ratio derivative spectra of the solutions of OFL at different concentrations were obtained by dividing each with the stored standard spectrum of the CEF (15 µg/mL) as shown in (Fig 1B). Wavelength 347.40 nm was selected for the quantification of OFL in CEF+ OFL mixture. Measured analytical signals at these wavelengths were proportional to the concentrations of the drugs over the selected concentration range. Calibration curves were prepared from the measured signals at the selected wavelength and concentration of the standard solutions. The concentrations of CEF (C_{CEF}) and OFL (C_{OFL}) in solution of tablets was calculated by using equations(1) and (2), respectively.

At 319.11 nm: C_{CEF} = (CEF Ratio derivative amplitude – 0.0006)/0.0806.... (1)
At 347.40 nm: C_{OFL} = (OFL Ratio derivative amplitude – 0.0429)/1.222.… (2)

Method B: Area Under Curve

For the simultaneous determination using the AUC method, suitable dilutions of the standard stock solutions (1000 µg/mL) of CEF and OFL were prepared separately in 0.1N HCl and further diluted with distilled water to make appropriate conc. range. The solutions of drugs were scanned in the range of 200-400 nm. The zero order overlain spectra are shown in Fig 2. For the method, sampling wavelength ranges selected for estimation of analytes were 277-279 nm (λ1-λ2) and 296-298 nm (λ3-λ4). Mixed standards were prepared and their integrated area under the curve were measured at the selected wavelength ranges [17-18]. Concentration of CEF and OFL in mixed standard and the sample solution were calculated using equation 3 and 4, respectively [19].

$C_{CEF} = A_2 \times a_{y1} - A_1 \times a_{y2} / a_{X2} \times a_{Y1} - a_{X1} \times a_{Y2}$ …… (3)
$C_{OFL} = A_2 - a_{X2} \times C_{CEF} / a_{Y2}$ …… (4)

Where,
a_{X1} (901.5) and a_{X2} (842.5) are the absorptivities of CEF at (λ1-λ2) and (λ3-λ4), respectively.
a_{Y1} (1737) and a_{Y2} (639.5) are the absorptivities of OFL at (λ1-λ2) and (λ3-λ4), respectively.
A_1 and A_2 are absorbances of mixed standard at (λ1-λ2) and (λ3-λ4) respectively. C_{CEF} and C_{OFL} are the concentrations in g/100 mL.

Recovery studies
The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 8 µg/mL, each of CEF and OFL.

Solution Stability
Method stability was checked by analyzing solution kept in fridge and at room temperature by both methods. Solution at room temperature was stable for 24 and 12 hours as tested by method A and method B, respectively (% RSD < 2). Solution in fridge were stable for 30 days and 15 days as tested by method A and method B, respectively (% RSD < 2).

Precision of the Method
Method repeatability was determined by six times repeatations of assay procedure. For intra-day precision method was repeated 5 times in a day and the average % RSD was determined. Similarly the method was repeated on five different days for inter-day precision and average % RSD was determined (Table 1). Precision of analyst was determined by repeating study by another analyst working in the laboratory.

Dissolution study
The dissolution study was carried out for the above combination and was validated. A calibrated dissolution apparatus (USP II) was used with paddles at 50 rpm and bath temperature maintained at 37 ± 1°C. Nine hundred milliliter freshly prepared and degassed 0.1N HCl solution was used as the dissolution medium. Six tablets were evaluated and dissolution sample were collected at 5, 10, 15, 20, 25, 30, 35, 40 and 45 min interval. At each time point, a 5 mL sample was removed from each vessel with replacement, it was filtered through Nylon filter (0.45µm, 25 mm) and 1.0 mL of filtrate was diluted to 10 mL with distilled water and analyzed by AUC method. Percentage release of CEF and OFL was calculated by using equations 5 and 6, respectively.

$$\text{CEF \% release} = (C_{CEF} \times 900 \times 10 \times 100)/ (1000 \times 200) \ldots\ldots. (5)$$
$$\text{OFL \% release} = (C_{OFL} \times 900 \times 10 \times 100)/ (1000 \times 200) \ldots\ldots. (6)$$

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. Using appropriate dilutions of standard stock solution the two solutions were scanned separately. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient are shown in Table 1. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy and

precision. Beer's law is obeyed in the concentration range of 5-25 µg/mL and 4-20 µg/mL for CEF and OFL for ratio derivative and AUC method, respectively, correlation coefficient was always greater than 0.999 for both the drugs. The proposed methods were also evaluated by the assay of commercially available tablets containing CEF and OFL. The results of formulation analysis are presented in Table 1. For CEF, the recovery study results ranged from 98.60 – 101.80 % and 98.75 – 100.2% for OFL by ratio derivative method and 98.16–100.4% for CEF and 98.89-100.21% for OFL by AUC method, respectively for both the formulations. Results of recovery studies are also shown in Table 2.

Table 1: Optical characteristics of the proposed methods and result of precision and formulation analysis

Parameter		Cefixime		Ofloxacin	
		Method A	Method B	Method A	Method B
wavelength (nm)		319.11	277-279	347.40	296-298
Beer's law limit (µg/mL)		5-25	4-20	5-25	4-20
Regression Equation*	Slope (m)	0.0806	-	1.2221	-
	Intercept (c)	0.0006	-	0.0429	-
Correlation coefficient		0.9998	-	0.9995	-
Precision (%RSD)	Repeatability (n=5)	0.68	0.76	0.59	0.82
	Intra-day (3x5 times	1.16	0.78	0.59	1.40
	Inter-day(3x5 days)	1.09	0.95	1.23	0.83
	Analyst	0.74	0.93	0.64	0.87
Formulation Analysis (%Assay, %RSD), n=6	TI	98.81% ± 0.32	100.8% ± 0.45	98.91% ± 0.29	101.2% ± 0.4
	TII	99.01% ± 0.46	100.9% ± 0.32	98.91% ± 0.51	101.72% ± 0.3

RSD = Relative Standard Deviation, Y = mX + c, where Y is the absorbance and X the concentration in micrograms per milliliter*

Table 2: Result of recovery studies of CEF and OFL by the proposed methods

Formulation studied	**Recovery Level**	**Recovery of**	**Amount Spiked (µg/mL)**	**% Mean Recovery, % RSD by n=6**	
				Method A	**Method B**
Formulation I	50%	CEF	4	99.60, 0.38	100.39 0.79
		OFL	4	98.75, 1.05	99.45, 0.97
	100%	CEF	8	98.63, 0.92	99.90, 1.03
		OFL	8	99.13, 1.72	98.89, 1.57
	150%	CEF	12	101.75, 0.74	100.05, 0.19
		OFL	12	99.66, 0.93	98.93, 1.34
Formulation II	50%	CEF	4	99.46, 0.53	100.02, 0.38
		OFL	4	100.2, 0.69	99.12, 1.05
	100%	CEF	8	99.47, 1.10	98.56, 0.92
		OFL	8	99.41, 0.80	100.20, 1.72
	150%	CEF	12	99.81, 1.52	98.16, 0.74
		OFL	12	99.52, 0.95	99.06, 0.93

The accuracy and reproducibility is evident from the data as results are close to 100 % and standard deviation is low. AUC method was applied for dissolution study and percentage release

during dissolution study was always greater than 80% within 45 minutes for both drugs for both tablet formulation under study (Fig. 3).

Method A involves dividing the spectrum of mixture into the standardized spectra for each of the analyte and first derivative of ratio spectrum was obtained which was independent of concentration of divisor. Method B involves formation and solving of simultaneous equation. Once the equations are formed, then only measurement of the integrated area of sample solution at two wavelength ranges and simple calculations are required and the method was successfully applied for dissolution study.

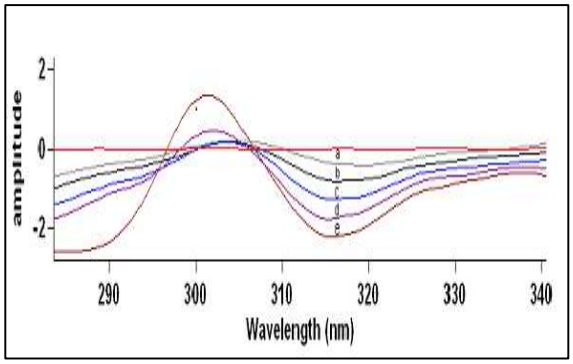

Fig.1A: First Derivative of ratio spectra of (a)5,(b)10,(c)15,(d)20,(e)25µg/ml of CEF when15µg/ml of OFL is used as divisor

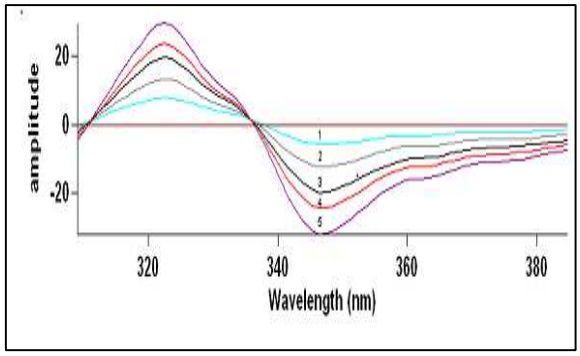

Fig.1B: First Derivative of ratio spectra of (1)5,(2)10,(3)15,(4)20,(5)25µg/ml of OFL When 15µg/ml of CEF is used as divisor

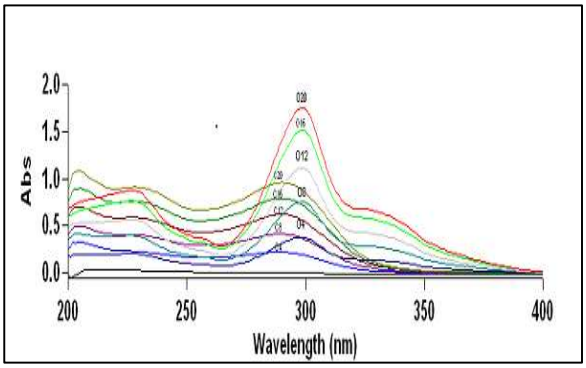

Fig. 2: Overlain spectra of CEF: (1) 4µg/mL; (2) 8 µg/mL; (3) 12 µg/mL;(4) 16 µg/mL;(5) 20 µg/mL; and OFL (A) 4 µg/mL;(B) 8 µg/Ml (C) 12 µg/mL;(D)16 µg/mL;(E)20 µg/mL in 0.1N HCl.

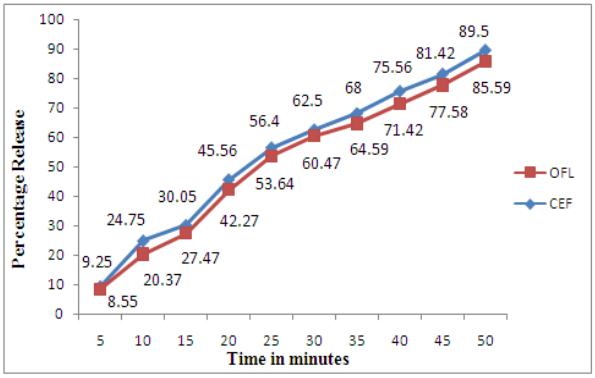

Fig.3:Dissolution profile of CEF and OFL tablet formulation by AUC method (n=6)

CONCLUSION

The validated spectrophotometric method employed here proved to be simple, economical, precise and accurate. Thus it can be used as IPQC test and for routine simultaneous determination of CEF and OFL in tablet dosage form. AUC method can be used to carry out dissolution study in combination tablet formulation.

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