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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF OFLOXACIN AND CEFEXIME FROM COMBINED ORAL DOSAGE FORM

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ABSTRACT

A Simple, accurate, sensitive, cost effective and specific spectrophotometric method was developed for the quantization estimation of Cefexime (CEF) and Ofloxacin (OFL) in solid dosage form. In simultaneous equation method both CEF and OFL shows maximum absorbance at 237.00 nm and 288.00 nm respectively in 0.1N NaOH. The linearity ranges for CEF and OFL was 10-50µg/ml. The percentage drug estimated in marketed preparation was found to be 99.61±0.1298 and 99.56±0.2371 for CEF and OFL. The method was validated for various parameters as per ICH guidelines and USP requirements. The accuracy of the method was assessed by recovery studies and was found to be 99.15 - 99.52% and 99.43 - 99.85% for CEF and OFL respectively. Therefore the proposed method can be used for routine analysis in quality control laboratories.

Key Words: Cefexime, Ofloxacin, Spectrophotometry, Validation.

INTRODUCTION

Cefexime (CEF) is an oral third generation cephalosporin antibiotic. Chemically, it is (6R,7R)-7-{[2-(2-amino-1,3-thiazol-4yl)-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 gonorrhea, otitis media, pharygitis, lower respiratory-tract infections such as bronchitis, and urinary tract infection. Ofloxacin (OFL) is a synthetic broad spectrum antibacterial agent. Chemically Ofloxacin is a fluorinated carboxy quinolone, is a racemate, (±)- 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. The chemical structure of Cefexime and Ofloxacin are shown in Figure 1 and 2 respectively. Both drugs are marketed as combined dosage formulation in the ratio of 1:1; CEF: OFL. Literature survey reveals that Cefexime can be estimated by spectrophotometry (Kasture VS *et al*., 2004; Wate SP *et al*., 2007), HPLC (Bhusari KP *et al*., 2009; Dhandapani B *et al*., 2010; Kathiresan K *et al*., 2009; Pasha K *et al*., 2010; Wankhede AR *et al*., 2010; Zendelovska D *et al*., 2003) and HPTLC methods individually or with any other drug in combination. However, there is no method was reported for simultaneous estimation of CEF and OFL in combined dosage form. Hence present work is to develop simple, accurate and reproducible method for simultaneous estimation of CEF and OFL in tablet formulation by spectrophotometry.

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MATERIALS AND METHODS

Instrumentation

UV–Visible Spectrophotometer (Shimadzu UV-

Visible spectrophotometer 1601) with 1 cm matched quartz cuvettes were used for all absorbance measurements. Electronic Weighing Balance (Citizen Analytical Balance) was used.

Reagents and chemicals

Standard Cefexime and Ofloxacin as gift sample were provided by Zim Laboratories Pvt. Ltd., Nagpur. The tablet of combined dosage of Cefexime and Ofloxacin were purchased from local market. All the chemicals were used of analytical grade.

Preparation of Stock Solution

Standard stock solutions CEF and OFL were prepared by dissolving 50mg of each drug in 0.1N NaOH. Stock solution of CEF and OFL were further diluted to get required concentration by using 0.1N NaOH.

Preparation of calibration curves

Appropriate dilutions of the standard stock solution were done separately to get 10, 20, 30, 40 & 50 µg/ml of CEF and OFL respectively. The absorption spectra were recorded between 200-400 nm. the absorbance were measured at 237.80 nm (λmax of CEF), 288.70 nm (λmax of OFL) and 247.91 nm (iso-absorbptive point). Beer's Lambert range for CEF and OFL were selected and working calibration curves for both the drugs were plotted separately. Overlain absorption spectra were recorded and a calibration curve was constructed by plotting absorbance *vs* concentration at each wavelength and regression coefficients were calculated (Figure 4-5).

Determination of absorptivity value of CEF and OFL

Appropriate dilution of the standard stock solution were done to get 10µg/ml of each CEF and OFL at 237nm (λmax of CEF), 288nm (λ_{max} of OFL). The absorptivity values of drug were determined at the selected wavelength. The absorptive values are the mean of six determinations.

Quantitative determination of CEF and OFL in tablet

20 tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Weighed tablet powder equivalent to 100 mg of CEF to 100 ml volumetric flask. 0.1 N NaOH added, ultrasonicated for 10min. and volume made to mark with 0.1 N NaOH. The solution then filtered through Whattman filter paper no.41. The filtrate was further diluted with 0.1 N NaOH to obtain concentration 10µg/ml of CEF and OFL. The concentration of both CEF and OFL were determined by measuring the absorbance of sample at 237.0 nm and 288.0 nm.

The concentrations of CEF and OFL were calculated by solving these simultaneous equations.

C x= (A1 ay2 - A2 Ay1) / (ax1 ay2 - ax2 ay1)……. (1)

C y = (ax1 A2 - ax2 A1) / (ax1 ay2 - ax2 ay1)…….. (2)

Where,

ax1 = Absorptivity of **CEF** at 237.0 nm

ax2 = Absorptivity of **CEF** at 288.0 nm

ay1 = Absorptivity of **OFL** at 237.0 nm

ay2 = Absorptivity of **OFL** at 288.0 nm

Cx and Cy are concentration of **CEF and OFL** respectively in the sample solution.A1 & A2 are the absorbance of the mixture at 237.0 nm and 288.0 nm respectively.

Validation of Developed Methods

The proposed methods have been statistically validated in terms of linearity, accuracy, precision, repeatability and reproducibility, limit of detection (LOD) and limit of quantification (LOQ) as per ICH Q2A guidelines (ICH, 2005).

a) Linearity

The linearity of the measurement was evaluated by analyzing different concentrations of the standard solution of CEF and OFL. For simultaneous equation, the Beer-Lambert's concentration range was found to be from 10-50µg/ml for both CEF and OFL.

b) Accuracy

To study of accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% & 120%). A known amount of drug was added to preanalyzed tablet powder and percentage recovery were calculated. The results of recovery studies were presented in Table.2

c) Precision

The reproducibility of proposed method was determine by performing tablet assay at different time interval (morning, afternoon and evening) on the same day (interday assay precision) and on three different days (interday precision). Result of intraday and interday precision is expressed in % RSD. Percent RSD for interday assay precision was found to be 0.852 (for CEF) and 0.923 (for OFL).

d) Specificity

Specificity study was performed by keeping the sample under various stressed conditions at 60°C and 50°C by adding 1 mL of 0.1N HCl, 0.1N NaOH and 3% H_2O_2 solutions.

e) Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of six calibration curves and average slope of six calibration curves.

LOD=3.3 X σ/S

LOQ= 10 X σ/S

Where, σ = Standard deviation of the response

S = Slope of the calibration curve.

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of CEF and OFL. In simultaneous equation

method, wavelengths selected for analysis were 237.00 nm (λmax of Cefexime) and 288.00 nm (λmax of Ofloxacin). The absorbances of the standard solutions were measured at 237.00 nm and 288.00 nm respectively. The linearity concentration range was 10-50µg/ml for both CEF and OFL.

The 385.1 and 104.6 are the absorptivity of CEF and 337.0 and 605.3 are absorptivity of OFL at 237.0 and 288.0 nm respectively. C_1 and C_2 are concentration of CEF and OFL. Absorptivity coefficient were calculated for both the drugs at selected wavelengths and substituted in equations for determining concentration of CEF and OFL in tablet. Percent label claim for CEF and OFL in tablet analysis, by this method was found to be 99.61% and 99.56% respectively. Standard deviation for the six determination of the tablet sample was found to be less than ±2.0 indicating precision of the method. Accuracy of the proposed method was ascertained by recovery studies and the results are expressed as % recovery. Percentage recovery by this method was found to be 99.15 - 99.52% and 99.43 - 99.85% for the CEF and OFL respectively, standard deviation and coefficient of variance was satisfactorily low, indicating the accuracy of method. The LOD was found to be 0.098µg/ml for CEF and 0.084µg/ml for OFL, LOQ was found to be 0.326µg/ml for CEF and 0.280µg/ml for OFL, respectively. Intra-day and inter-day precision studies were carried out by analyzing tablet powder at different time interval on same day and on three different days, respectively. Based on the results obtained, it is found that the proposed method is accurate, precise, reproducible, & economical and can be employed for routine quality control of Cefexime and Ofloxacin in combined dose tablet formulation.

Table 1. Results of assay of tablets by simultaneous equation method

Method	Label claim(mg/tab)		Amount found (%)*		Standard deviation	
	CEF	OFL	CEF	OFL	CEF	OFL
Simultaneous equation	100	100	99.61%	99.56%	±0.1298	±0.2371

***Average of six determination*

Table 2. Results of Recovery Studies

Level of %Recovery	Mean % recovery		± SD		% RSD	
	CEF	OFL	CEF	OFL	CEF	OFL
80%	99.52	99.85	0.356	0.562	0.357	0.562
100%	99.15	99.56	0.423	0.433	0.426	0.434
120%	99.26	99.43	0.389	0.512	0.391	0.514

Mean of three estimations ,SD is Standard Deviation of n=3 observations, RSD is Relative Standard Deviation

Table 3. Results Specificity studies

S.No.	Conditions	% Labeled Claim of CEF	% Labeled Claim of OFL
1	Normal	99.52	99.83
2	Acid	65.53	86.25
3	Alkali	99.32	99.64
4	Oxide	95.23	93.52
5	Heat	97.23	98.52

Mean of three estimations

Table 4. Results of validation Parameters

Parameters	CEF	OFL
Linearity Range (µg/ml)	10 -50	10 - 50
Regression Equation (y = mx+c)	y=0.038x±0.0148	y=0.0579x±0.074
Correlation Coefficient (r2)	0.9996	0.9983
LOD (µg/ml)	0.098	0.084
LOQ (µg/ml)	0.326	0.280
Analysis of tablets (% Assay)	99.61%	99.56%
% Recovery	99.15 - 99.52	99.43 - 99.85
Intra Day Precision (% RSD)	0.932	0.982
Inter Day Precision (% RSD)	0.852	0.923
Repeatability (% RSD)	0.130	0.238

CEF is Cefexime, OFL is *Ofloxacin*, y = mx+c where y is *absorbance*, m is *slope*, c is *intercept*, LOD is *Limit of Detection*, LOQ is *Limit of Quantitation*, RSD is *Relative Standard Deviation*

Figure 1. Chemical structure of Cefexime (CEF)

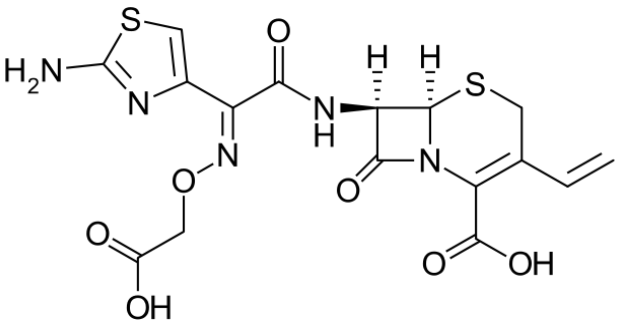


Figure 2. Chemical structure of Ofloxacin (OFL)

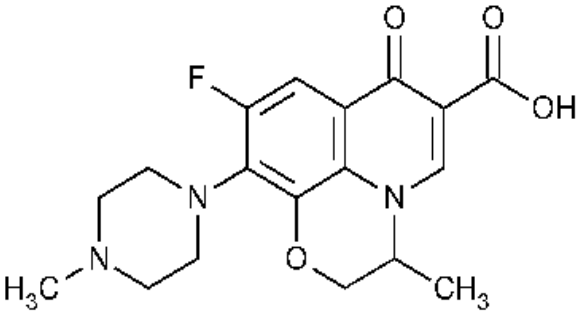


Figure 3. Overlain Spectra of Cefexime and Ofloxacin

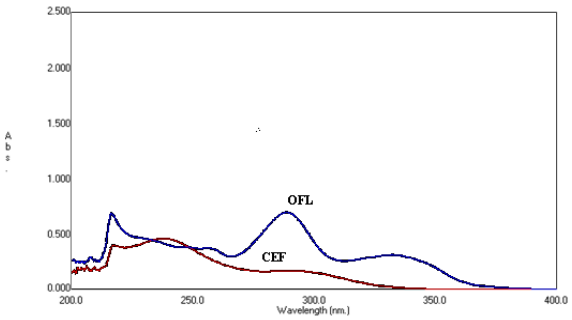


Figure 4. Calibration Curve for Cefexime

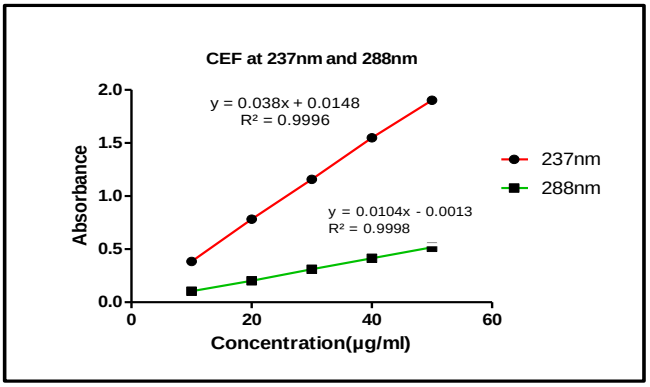
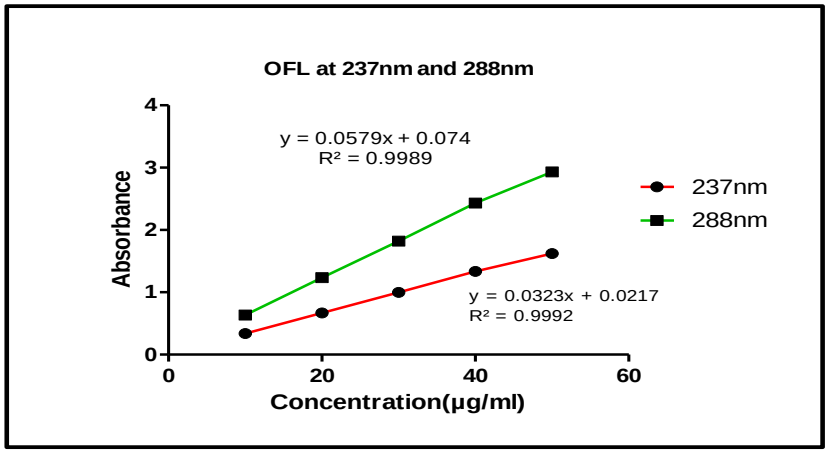


Figure 5. Calibration Curve for Ofloxacin



CONCLUSION

The experimental results demonstrate that the proposed UV-Spectrophotometric method using simultaneous equation method is simple, rapid, sensitive, accurate, precise and economical. Thus this method can be used for the determination of Ofloxacin and Cefexime either in bulk or in the combined solid oral dosage form. The excipients usually present in the pharmaceutical formulation did not interfere with determination of Ofloxacin and Cefexime. The developed method can be successfully used for routine quality control of Ofloxacin and Cefexime in their combined dosage form.

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