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Research Article

# DETERMINATION OF CEFIXIME AND OFLOXACIN BY RATIO SPECTRA AND ZERO CROSSING DIFFERENCE SPECTROPHOTOMETRY

**SIVAKUMAR CH.V*[1], SHUBHAM GUPTA[1], AMERENDRA K. CHANDAN[1], CHANDRAKALA GUNTURU[1], MEERA INDRACANTI[1]**

[1]Instrumental Methods of Analysis, Department of Biotechnology, ICFAI University, Rajawala Road, Dehradun, UttraKhand, India. Email: siva.bioteck@gmail.com

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**ABSTRACT**

Two new, simple, economical, reliable, precise and accurate Spectrophotometric methods of simultaneous determination of Cefixime and Ofloxacin in combined binary mixture tablet dosage form have been developed. First method was based on ratio spectra derivative spectrophotometry, and the second method is zero-crossing difference spectrophotometry. The amplitudes in the first order derivative of the resultant ratio spectra at 270.5 nm and 296.7 nm were selected to find out cefixime and ofloxacin respectively. In the second method measurements of absorbance were carried out at Zero-crossing wavelengths 257.11 nm and 284.07 nm by zero-crossing difference spectrophotometric method to determine the cefixime and ofloxacin respectively. Beer's law was obeyed in the concentration range of 5-30 µg/mL for Cefixime and 4-26 µg/mL, 4-16 µg/mL for ofloxacin by using ratio derivative method and zero-crossing difference spectrophotometric method. These methods passes F test and t test. The two methods were validated statistically by performing recovery studies.

**Keywords:** Cefixime, ofloxacin, Ratio spectra derivative spectrophotometry, Zero-crossing difference spectrophotometry.

## INTRODUCTION

Cefixime Trihydrate (CEF) chemically is (6R, 7R)-7-[[(z)-2-(2-aminothiazol-4-yl) 2-[(carboxy methoxy) imino] acetyl]-amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2ene-2-carboxylic acid[1]. It is a oral third generation of cephalosporin and is used as an antibacterial and especially against gram negative, gram positive and anaerobic bacteria pathogens including β- lactamase producing strains. It consists of high affinity for penicillin binding proteins with deceitful site of activity. It acts by inhibition of bacterial cell-wall synthesis. It is clinically used in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis and urinary-tract infections[2]. Literature survey revealed the estimation methods of cefixime trihydrate or with other drugs by HPLC[8,9], calorimetric method[7], flow injection analysis[10], and HPTLC[11].

Ofloxacin[2] (OFL) a fluorinated carboxyaquinolone, chemically 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. It is a synthetic broad spectrum antibacterial agent official in BP[3], USP[4] and EP[5]. Ofloxacin have much greater antibacterial activity towards urinary tract infections. It acts by inhibiting DNA gyrase of microorganisms. Literature survey reveals UV spectrophotometric method, atomic absorption spectrometry[12], spectroflurometry, HPLC[13] and microbiological method[14] for its determination.

Literature survey reveals no ratio spectra derivative[15] and zero-crossing difference[16] UV-spectroscopic methods were reported for this combination in tablet dosage form. These two methods are simple, rapid precise and economic and have been developed for the determination of CEF and OFL in combined pharmaceutical dosage form.

Measurement of through UV-Vis spectrophotmetric is subjected to interference from excipients used in formulation. Derivative spectrometry is a useful analytical technique to interpret the quantitative information from spectra of mixtures consists of two or more components with overlapped UV spectra, and for eliminating interference from the formulation matrix by using the zero-crossing technique. Furthermore, ratio-spectra derivative spectrometry has been found to be useful in the estimation of drugs in their mixtures by suppressing the matrix effects. In 1990, Salinas et al[18] developed the ratio spectra derivative spectrophotometry for the resolution of binary mixtures.

In the ratio spectra derivative method the spectrum of mixture was divided by the standardized spectra of each of the analyte and deriving the ratio spectra that is independent of the concentration of analyte used as divisor. The ratio spectra of various CEF standards at increasing concentrations were obtained by dividing each with the stored spectrum of the standard solution of OLF (12 ug/mL, scaling factor 4) and the first order derivative of these spectra traced with the interval of $\Delta\lambda$ =4nm. Wavelength 270.5 nm was selected for the quantification of CEF in CEF and OFL mixture. The ratio first order derivative spectra of the solutions of OFL at various concentrations were obtained by dividing each with the stored standard spectrum of the CEF (16 ug/mL, scaling factor 4) and the first derivative of these spectra traced with the interval of $\Delta\lambda$=4nm. Wavelength 296.7 nm was selected for the quantification of OLF in CEF and OLF mixture. Measured absorbance at these wavelengths is proportional to the concentrations of the drugs. The quantity of CEF and OFL in tablets was calculated using the following equations:

$$C_{CEF} = d/d\lambda\ [A_{CEF}/A_{OFL}]\text{-}(C) / (m) \quad \ldots\ldots\ldots\ldots (1)$$

$$C_{OLF} = d/d\lambda\ [A_{OFL}/A_{CEF}]\text{-}(C) / (m) \quad \ldots\ldots\ldots\ldots (2)$$

The method of zero-crossing difference spectrophotometry was based upon the measurement of a absorbance difference ($\Delta A$) which can be induce by changing the pH of solvent medium of two equimolar solutions of Cefixime and Ofloxacin in phosphate buffer (pH 9) solution against their chloride buffer (pH 2) solution as blank[17]. The choice of the optimum wavelength is based on the fact that the contribution of each component exhibited maximum absorbance. Therefore, a measurement of absorbance from difference spectra was carried out at zero-crossing wavelength at 257.07 nm for CEF and 284.07 nm for OFL.

## MATERIALS AND METHODS

A Perkin Elmer Double beam UV-visible spectrophotometer (Model Lambda 25) with 10-mm Matched quartz cells, bandwidth 1 nm was used for spectral measurement. The pH measurements were made with Systronics pH meter in combination with a calomel glass electrode. Pure drug samples of Cefixime and Ofloxacin (Roorkee Research Laboratories., Roorkee, India.) were used having 99 % and 99.8 % purity, respectively. Chloride buffer, pH 2 and phosphate buffer, pH 9 were used as solvent for zero-crossing difference spectrophotometric analysis, and 0.1 NaOH was used as solvent for ratio spectra first-derivative spectrophotometric method. All reagents used were of analytical grade and used without any further purification. Pure water was purified by deionized and filtered by Millipore system (Sartorius, USA) using 0.2 um membrane filter. Tablets were procured locally.

**Ratio Spectra Derivative Method**

**Preparation of standard stock solutions**

Standard stock solutions of pure drug were prepared by dissolving 10 mg of each pure drug CEF and OFL in 100 mL of 0.1 N NaOH using separate volumetric flask. The working solutions of these drugs were prepared by dilution of the respective stock solution with 0.1 N NaOH.

**Calibration Graph**

The working standards were prepared by using appropriate volumes of stock solution in the range of 5-30 µg/mL CEF and 4-26 µg/mL of OFL respectively. The ratio first order derivative spectra of the solutions of CEF at various concentrations were obtained by dividing each with the stored standard spectrum of the standard solution of OFL (12 µg/mL, Scaling factor 4), and the absolute values of first order derivative of these spectra were traced with the interval of Δλ=4 nm. Wavelength 270.5 nm was selected for the quantification of CEF in CEF and OFL mixture. The ratio first order derivative spectra of the solutions of OFL at various concentrations were obtained by diving the standard spectrum of standard solution of CEF (16µg/mL, Scaling factor 4) and the absolute values of first order derivative of these spectra were traced with interval of Δλ=4 nm. Wave length 296.07 was selected for the quantification of OFL in CEF and OFL mixture. The statistical parameters of the calibration graph were calculated.

**ZERO-CROSSING DIFFERENCE METHOD**

For the preparation of standards 10 mg of each drug were weighed accurately and dissolved in minimum quantity of methanol, and final volume were made with water in a 100mL volumetric flask. Further dilutions were made in respective buffer solutions as (a) CEF 20 ug/mL in acidic pH 2 with a maximum at 285.08 nm, (b) CEF 20 ug/mL in alkaline pH 9, (c) OFL 12 ug/ mL in acidic pH 2 with a maximum at 293 nm (d) OFL 12 ug/mL in alkaline pH 9 with a maximum at 288 nm and mixture (e, f) in acidic (20:15 ug/mL of CEF and OFL) in acidic pH 2 and in alkaline pH 9 and zero order spectra were recorded.

**Calibration graph**

Two series of 10mL equimolar solutions of mixtures each of CEF and OFL in chloride buffer pH 2 and phosphate buffer pH 9 were prepared. In the first series set the concentration of CEF (20 ug/mL) was constant and varying concentration of OFL (4-16 ug/mL). The second series consists of constant concentration of OFL (12 ug/mL) and varying concentration of CEF (5-30 ug/mL). Calibration graph of difference spectra was used for the calculation of results of CEF and OFL and statistical parameters of calibration were calculated.

**Preparation of Tablet sample solution**

Ten tablets (CEFIX-O) were weighed and ground to fine powder. From this sample equivalent to 20 mg of CEF was dissolved in minimum quantity of methanol and diluted up to 100mL with 0.1N NaOH in a volumetric flask (ratio-spectra method) and diluted with buffer acidic pH 2 and alkaline pH 9 (zero-crossing difference method) stirred for about 10 min. The solution was filtered through Whatman filter paper no.41. Results of CEF and OFL were computed from the graph.

**Recovery studies**

The accuracy of the proposed methods was checked by recovery studies with the addition of standard drug solution to pre-analyzed sample solution at three different concentration levels (60 % and 80 %) within the range of linearity for both the drugs.

**RESULTS AND DISCUSSION**

**For ratio spectra derivative method**

Zero-order spectra of pure drugs were found to be overlapping making simultaneous determination difficult. It can be seen that the absorption spectra of CEF and OFL are much overlapped results unreliable in direct absorbance measurement method.

Zero-order spectra of CEF (20ug/mL), OFL (15 ug/mL) and their mixture in 0.1 N NaoOH were recorded, which is shown in figure 1.

**Table 1: Optical Characteristics of linearity for CEF and OFL by Zero order spectrophotometric method**

| Method Parameter | | CEF | OFL |
|---|---|---|---|
| $\lambda_{max}$ (nm) | | 236 | 288 |
| Beer's law limit (µg/mL) | | 05-30 | 4-24 |
| Correlation Coefficient ($r^2$) | | 0.998 | 0.993 |
| LOD (µg/mL) | | 0.22 | 0.18 |
| LOQ (µg/mL) | | 0.67 | 0.55 |
| Regression equation (Y*) | | | |
| a) | Slope | 0.104 | 0.08 |
| b) | Intercept | 0.001 | 0.008 |

*Y*= a X + b* where Y is absorbance and X is concentration in µg/mL

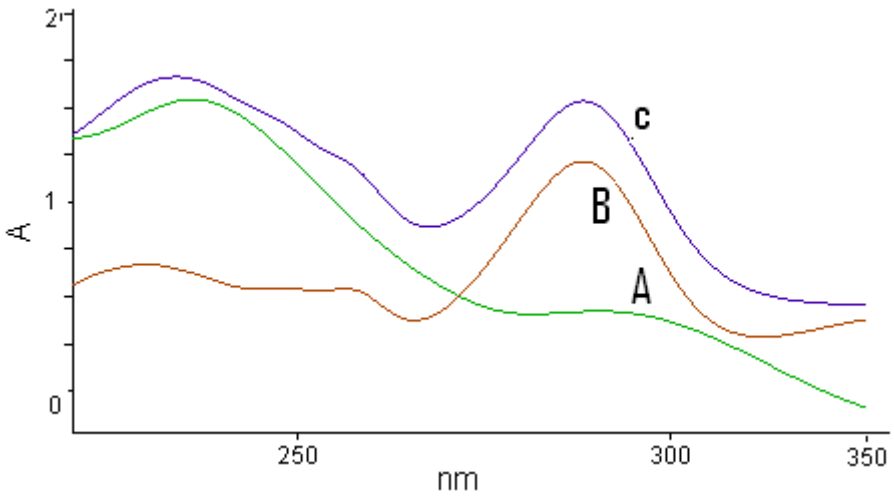



**Fig. 1: Zero-order spectra of a) CEF (20 µg/mL), b) OFL (15 µg/mL), c their mix in 0.1 N NaOH**

The linearity of pure drug was found to be 5-30 µg/mL for CEF and 4-26 µg/mL for OFL. The correlation coefficients 0.998 for both the drugs show good precision for linearity.

The ratio and ratio derivative spectra of the solutions of CEF at different concentrations were obtained by dividing each with the stored standard spectrum of the OFL (12 µg/mL, scaling factor 4),

and the first derivative of these spectra were traced with the interval of Δλ=4 nm. Wavelength 270.5 nm was selected for the quantification of CEF in CEF and OFL mixture. The ratio and ratio derivative spectra of OFL at different concentrations were obtained by dividing each with stored standard of CEF (16 µg/mL, scaling factor 4), and the first derivative of these spectra were traced with the interval of Δλ=4 nm. Wave length 296.7 nm was selected for the quantification of OFL in CEF and OFL mixture. The correlation coefficients 0.998 for CEF and 0.999 for OFL show good precision for calibration.

**Table 2: Optical Characteristics of linearity for CEF and OFL by Ratio Derivative and Difference spectrophotometric method**

| | Method | | | |
|---|---|---|---|---|
| | Ratio Derivative method | | Difference method | |
| **Parameter** | **CEF** | **OFL** | **CEF** | **OFL** |
| $\lambda_{max}$ (nm) | 270.5 | 296.7 | 257.17 | 284.07 |
| Beer's law limit (µg/mL) | 5-30 | 4-26 | 5-30 | 4-26 |
| Correlation Coefficient ($r^2$) | 0.998 | 0.991 | 0.99 | 0.989 |
| LOD (µg/mL) | 0.079 | 0.06 | 0.069 | 0.054 |
| LOQ (µg/mL) | 0.24 | 0.18 | 0.22 | 0.209 |
| Regression equation (*Y*)* | | | | |
| a) Slope | 0.0032 | 0.010 | 0.0018 | 0.012 |
| b) Intercept | 0.014 | 0.001 | 0.014 | -0.005 |

*Y* = aX + b*, where Y is the absorbance and X the concentration in µg/mL

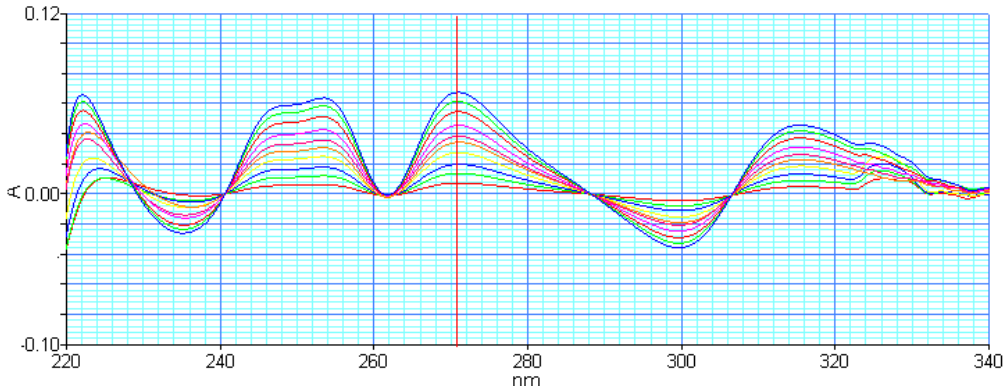



**Fig. 2: First-order ratio spectra of solutions of CEF (05–30 µg/mL) when 12 µg/mL solution of OFL is used as divisor (Δλ=4 nm)**

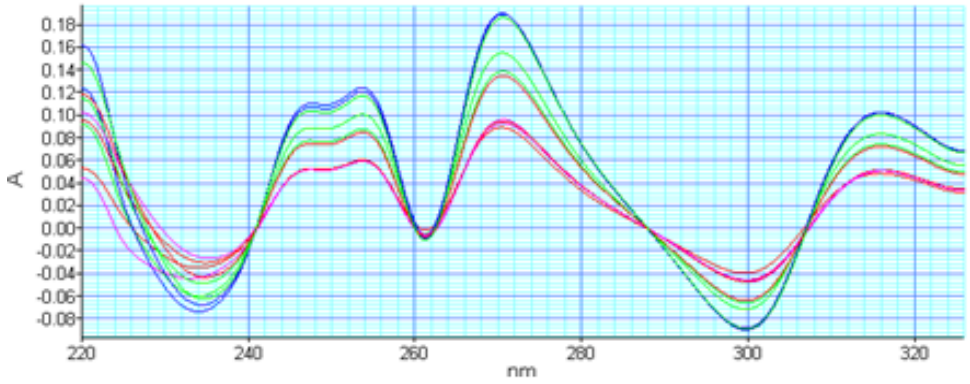
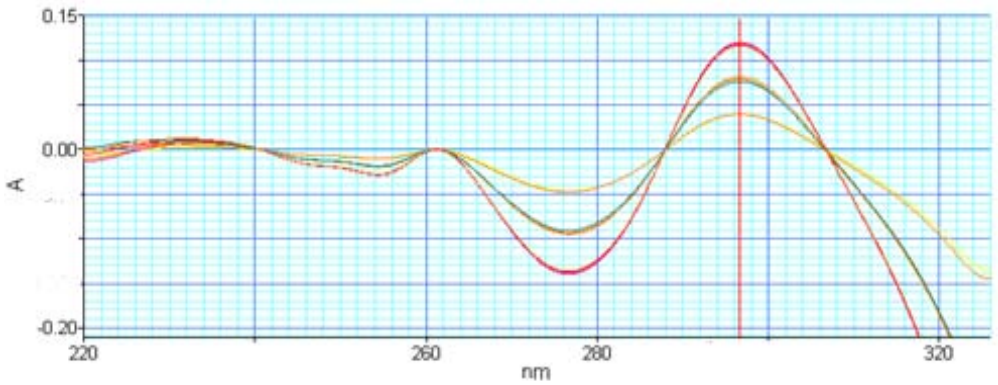



**Fig. 3: First-order ratio spectra of OFL (04–26 µg/mL) when 16 µg/mL solution of CEF is used as divisor (Δλ=4 nm)**



The influence of other parameter scan speed was studied to optimize the signal of derivative ratio spectra to give good selectivity and higher sensitivity in detection. Medium scan speed 480 nm/min was chosen throughout the work. The other parameter was the concentration of divisor in order to test the effect on calibration graph. The change in concentration of divisor effects the calibration graph due to change in minima and maxima but the wavelength remains same. The changes in Δλ affect the zero crossing wavelengths which affect the size and shape of peak and position of the peak finally the optimized Δλ was chosen.

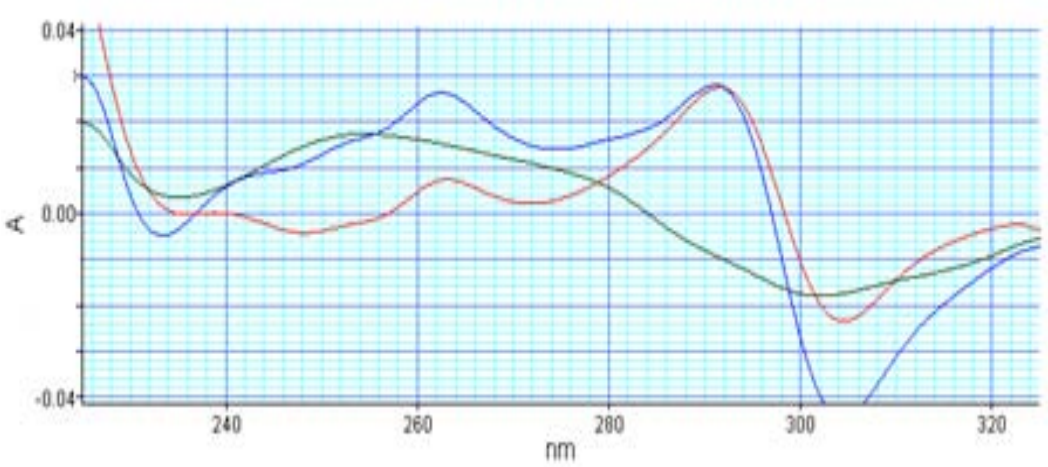



**Fig. 4: Difference spectra of A) CEF 20 µg/mL), B) OFL (15 µg/mL) and mixture C (20:15 µg/mL) in acidic (pH 2) vs alkaline (pH 9) pH solution**

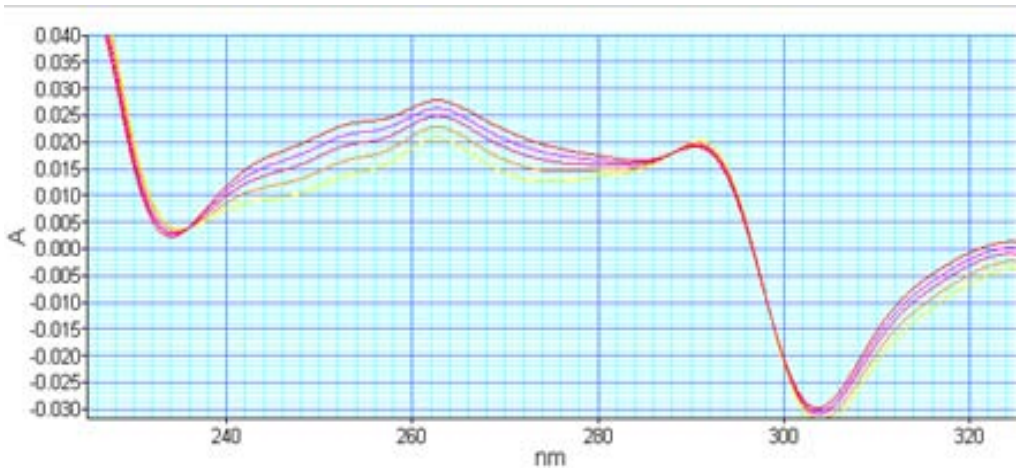



**Fig. 5: Difference spectra of CEF in mixture (15 µg/mL OFL and 5-30 µg/mL CEF) in acidic (pH 2) vs alkaline (pH 9) pH solution**

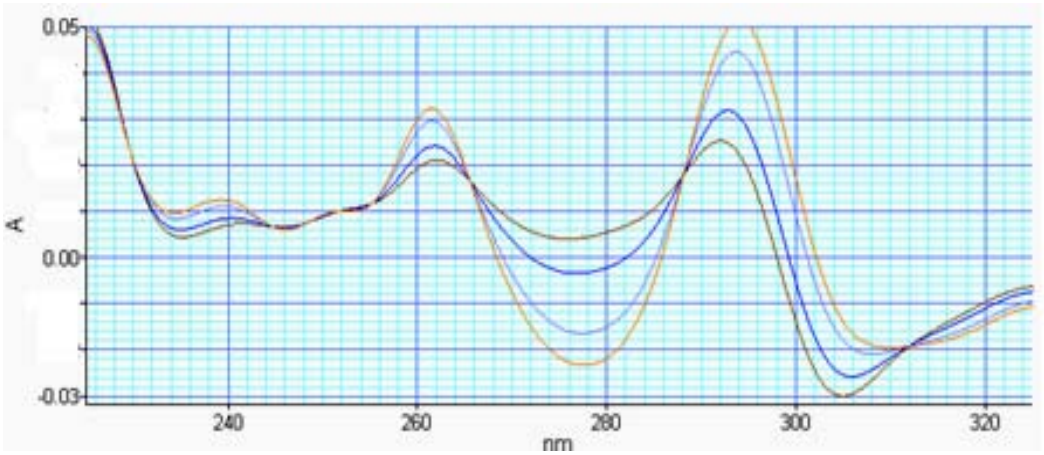



**Fig. 6: Difference spectra of OFL in mixture (20 µg/mL CEF and 4-26 µg/mL OFL) in acidic (pH 2) vs alkaline (pH 9) pH solution**

**For zero-crossing method**

Zero order Absorption spectra of following (a) CEF 20 µg/mL in acidic pH 2 with maximum at 285.08 nm and (b) CEF 20 µg/mL in alkaline pH 9 with a maximum at 236.17 nm, (c) OFL 12 µg/mL in acidic pH 2 with maximum at 288 nm, (d) OFL 12 µg/mL in alkaline pH 9 with maximum at 293 nm and (e, f) mixture (20:12 µg/mL) in acidic pH 2 and alkaline pH 9 solutions were recorded. The linearity was found to be in concentration range of 5-30 µg/mL and 4-16 µg/mL for CEF and OFL respectively. The correlation coefficients 0.996 for CEF and 0.998 for OFL show good precision for linearity. (Table I). The difference spectra of calibration graph for CEF and OFL are depicted in Figs 5 and 6. The correlation coefficients 0.997 for CEF and 0.998 for OFL show good precision for calibration (Table II).

The method was estimated by performing assay of commercially available tablet formulations containing CEF and OFL. Both ratio spectra and zero crossing method results of the assay are shown in table III. The percent relative standard (RSD) values for recovery study was within the limit and show good precision and reproducibility. The results of recovery for both the drugs are shown in table IV.

**Table 3: Results of Analysis of Commercial Formulation for CEF and OFL by Difference and Ratio Derivative Method**

| Method for estimation | Ratio derivative method | | Difference method | |
|---|---|---|---|---|
| | CEF | OFL | CEF | OFL |
| Label claim | 200 | 200 | 200 | 200 |
| % of Label claim estimated | 99.86 | 100.4 | 100.1 | 101.25 |
| Standard deviation | +0.59 | +0.361 | + 0.668 | + 0.875 |

Average of six determinations

**Table 4: Recovery studies of CEF and OFL by Ratio derivative and Difference method**

| S. No. | Name of the Compound | Percentage of the drug Substance added (%) | Drug added µg/mL | Mean recovery | Standard Deviation |
|---|---|---|---|---|---|
| | CEF | 60 | 6 | 99.67 | 0.66 |
| Method-I | | 80 | 8 | 99.89 | 0.36 |
| | OFL | 60 | 6 | 100.2 | 0.54 |
| | | 80 | 8 | 99.87 | 0.89 |
| Method-II | CEF | 60 | 6 | 98.99 | 0.66 |
| | | 80 | 8 | 99.9 | 0.36 |
| | OFL | 60 | 6 | 99.2 | 0.54 |
| | | 80 | 8 | 100.87 | 0.89 |

Average of three determinations

**Detection and quantification Limits**

According to recommendations of ICH[19] the limit of detection, LOD =3.3 σ/s, where σis the standard deviation of the intercept of the regression line and s is the sensitivity, namely the slope of the calibration graph. On the other hand, the limit of quantification, LOQ is defined as 10σ/s. detection and quantification limits of the drugs using the proposed methods were calculated and are presented in table 2.

Both the methods were evaluated by performing *F* test and *t* test. The calculated *F* value 0.298 for CEF and 0.570 for OFL are less then theoretical *F* (0.05, 5) =4.28. We can conclude that there is no significant difference in the precision of the two methods, and the calculated *t* values 0.48 0.81 for CEF and 0.61 0.89 OFL are less than the theoretical *t* (0.05, 5) =2.447 value showing there was no significant difference in precision of two proposed methods.

**CONCLUSION**

The validated ratio spectra derivative and zero-crossing difference spectrophotometric methods are simple, economical, rapid, precise, and accurate and successfully adopted for simultaneous quantification of binary mixture of CEF and OFL. Thus, it can be used as alternative method for routine simultaneous determination of CEF and OFL in combined pharmaceutical tablet dosage form. The ratio spectra derivative method has greater sensitivity.

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**REFERENCES**

1. Maheshwari RK. Spectrophotometric determination of cefixime in tablets by hydrotropic solubilization phenomenon. *The Indian pharmacist*. 2005; 36: 63-68.
2. Budavari S. Eds. In. The Merck Index. Merck & co., Inc; 13th Ed: Whitehouse Station, NJ; 2001. p.1213 and 1229.
3. British pharmacopoeia. Licensing division HMSO, Norwich; 2003. p. 357.
4. United States Pharmacopoeia. United States Pharmacopoeial Convention, Inc. Rockville; 2004. p.1335.
5. European Pharmacopoeia. EDQM, Council of Europe. 5th Ed. Strasbourg, France. 2005. p.2131.
6. Sweetman S.C. Martindale. The Complete Drug Reference. 32nd Edition. Pharmaceutical Press, London. 1999, p.165-166.
7. Rajnish Kumar, Pinderjit Singh, Harinder Singh. Development of colorimetric method for the analysis of pharmaceutical Formulation containing both ofloxacin and cefixime. International journal of pharmacy and pharmaceutical sciences. 2011; 3:152-155.
8. Arshad HM, Gauhar S, Bano R, Muhammad IN. Development of HPLC-UV method for analysis of cefixime in raw materials and capsule. Jordan journal of pharmaceutical sciences. 2009; 2; (1):53-65.
9. Kapil S. Khandagle, Santosh V. Gandhi, Padmanbh B. Deshpande, And Nilesh V.Gaikwad. A simple and sensitive RP-HPLC for simultaneous estimation of cefixime and ofloxacin in combined tablet dosage form. International journal of pharmacy and pharmaceutical sciences. 2011; 3, (1):46-48.
10. Al-Momani I. F. Spectrophotometric determination of selected cephalosporins in drug formulations using flow injection analysis. Journal of Pharmaceutical and Biomedical Analysis. 2001; 25:751–57.
11. Khandagle K. S, Gandhi S.V, Deshpande P. B, Gaikwad, V.N. High performance Thin Layer Chromatographic determination of cefixime and ofloxacin in combined tablet dosage form. Journal of Chemical and Pharmaceutical Research, **2010**, 2, 5, 92-96.
12. Hesham Salem. Spectroflurometric, Atomic absorption spectrometric and spectrophotometric determination of some

fluroquinolones. American Journal of Applied Sciences.2005; 2: 719-729.

13. Arjekar AP, Kapadia US, Raj SV and Kunjir SS. Quantitative determination of lomefloxacin, ofloxacin, pefloxacin and enrofloxacin in pharmaceutical dosages, bulk drug and processes monitoring of enrofloxacin by HPLC-RP. Indian Drugs.1996; 33: 261-266.
14. Silveria EvL and Schapoval, EES.Microbiological assay for determination of ofloxacin injection. J. Pharm. Biomed. Anal. 2002; 1-2; 91-96.
15. Sayed AY, Salem NA. Recent development of derivative spectrophotometry and their analytical applications. Analytical Sci. 2005; 21, 595.
16. Basilio M. Determination of Anafranil in pharmaceuticals by difference and difference-derivative spectrophotometric method. Analytical Letters. 1996 29(9):1551–62.
17. Beckett SJB. Practical pharmaceutical chemistry, 4th edition, part 2. Delhi: CBS Publishers & Distributors; 2004. p. 293–96.
18. Salinas, F.; Berzas Nevado, J.-J.; Espinosa Mansilla, A. A new spectrophotometric method of quantitative multicompnent analysis, resolution of mixtures of salicylic and salicyluric acids. Talanta **1990**, *37*(3), 347.
19. Guidance for industry; Q2B validation of Analytical procedures: Methodology; International Conference on Harmonization (ICH), November 1996, Please visitat http:www.fda.gov/evder/guidance/1320fnl.pdf.