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# DEVELOPMENT AND VALIDATION OF SPECTROPHOTO-METRIC METHOD FOR SIMULTANEOUS DETERMINATION OF OFLOXACIN AND CEFPODOXIME PROXETIL IN TABLETS

**Sanket A. Patel*[1], Satish A. Patel[1]**

**1.** Center for Health Science Studies, Ganpat University, Kherva, Mehsana, Gujarat, India.

## ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of Ofloxacin and Cefpodoxime proxetil in combined tablet dosage form. The method is based on the simultaneous equations for analysis of both the drugs using methanol as solvent. Ofloxacin has absorbance maxima at 297 nm and cefpodoxime proxetil has absorbance maxima at 236.2 nm in methanol. The linearity was obtained in the concentration range of 2-12 µg/ml and 4-24 µg/ml for Ofloxacin and Cefpodoxime proxetil, respectively. The concentrations of the drugs were determined by using simultaneous equations at both the wavelengths. The mean recovery was 99.63 ± 0.47 and 99.57 ± 0.36 for Ofloxacin and Cefpodoxime proxetil, respectively. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found. The suitability of this method for the quantitative determination of Ofloxacin and Cefpodoxime proxetil was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of Ofloxacin and Cefpodoxime proxetil in pharmaceutical tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

**Key words:** Cefpodoxime proxetil, Ofloxacin, recovery, simultaneous equations method, tablet, validation.

*Corresponding Author Email: san24_9@yahoo.com

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## INTRODUCTION

Ofloxacin (OFLO) is chemically 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[1] (Figure 1) is a fluoroquinolone antibacterial used in the treatment of chalmydia or chlamydophila infections including nongonococcal urethritis and in mycobacterial infections such as leprosy[2]. It is official in IP, BP and USP. IP[3], BP[4] and USP[5] describe potentiometry method for its estimation. Literature survey reveals spectrofluorimetric[6, 7], HPLC[8, 9] and chemiluminescence[10] methods for determination of OFLO in pharmaceutical dosage forms as well as in biological fluids. Literature survey also reveals spectophotometric[11], RP-HPLC[12] and HPTLC[12] methods for determination of OFLO with other drugs in combination. Cefpodoxime proxetil (CEFPO) is chemically 1-(isopropoxy carbonyloxy) ethyl(6R,7R)-7-[2-(2-amino-4-thiazolyl)-(z)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate[13] (Figure 2) is a third generation cephalosporin

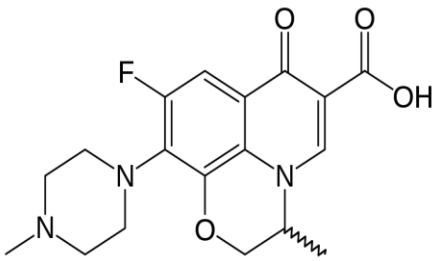


**Figure 1:** Structure of Ofloxacin (OFLO)

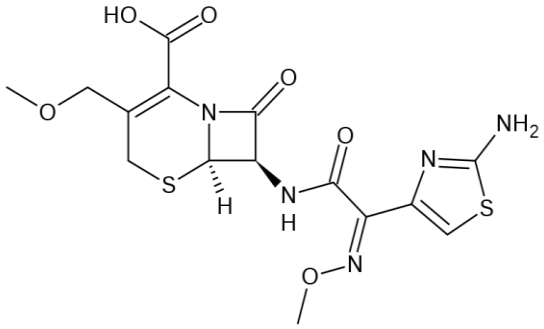


**Figure 2:** Structure of Cefpodoxime (CEFPO)

antibiotic used for infections of the respiratory tract, urinary tract and skin and soft tissues[14]. Cefpodoxime proxetil is official in IP and USP. IP[15] and USP[16] describe liquid chromatography method for its estimation. Literature survey reveals HPTLC[17] method for the determination of CEFPO. Literature survey also reveals RP-HPLC[18] and spectophotometric[19] methods for determination of CEFPO with other drugs in combination. The combined dosage forms of OFLO and CEFPO are available in the market and used as antibacterial drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of OFLO and CEFPO in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or other method for simultaneous estimation of OFLO and CEFPO in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on simultaneous equations for simultaneous estimation of both drugs in their combined tablet dosage form.

# MATERIALS AND 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

OFLO and CEFPO bulk powder was kindly gifted by Acme Pharmaceuticals Ltd. Ahmedabad, Gujarat, India. The commercial fixed dose combination product was procured from the local market. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

## Preparation of standard stock solutions

An accurately weighed quantity of OFLO (10 mg) and CEFPO (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 OFLO (100 µg/ml) and CEFPO (100 µg/ml).

## Methodology

The standard solutions of OFLO (10 µg/ml) and CEFPO (10 µg/ml) were scanned separately in the UV range of 200-400 nm. Maximum absorbance was obtained at 297 nm and 236.2 nm for OFLO and CEFPO, respectively. These two wavelengths can be employed for the determination of OFLO and CEFPO without any interference from the other components in their combined formulations.

## Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[20]

## Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-12 µg/ml for OFLO and 4-24 µg/ml for CEFPO. Accurately measured standard solutions of OFLO (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) and CEFPO (0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbances of the solutions were measured at 297 and 236.2 nm against methanol as blank. The calibration curves were

constructed by plotting absorbances versus concentrations and the regression equations were calculated.

**Method precision (repeatability)**

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions ($n$ = 6) for OFLO and CEFPO (10 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method.

**Intermediate precision (reproducibility)**

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of OFLO and CEFPO (4, 8, 12 µg/ml for OFLO and 8, 16, 24 µg/ml for CEFPO). The result was reported in terms of relative standard deviation (% RSD).

**Accuracy (recovery study)**

The accuracy of the method was determined by calculating recovery of OFLO and CEFPO by the standard addition method. Known amounts of standard solutions of OFLO and CEFPO were added at 50, 100 and 150 % level to prequantified sample solutions of OFLO and CEFPO (4 µg/ml for each drug). The amounts of OFLO and CEFPO were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times.

**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[20].

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

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

Where, $\sigma$ = the standard deviation of the response and S = slope of the calibration curve.

**Analysis of OFLO and CEFPO in combined tablet dosage form**

Twenty Tablets were weighed and powdered. The powder equivalent to 10 mg of OFLO and 10 mg of CEFPO was transferred to a 100 ml volumetric flask. Methanol (50 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. This solution is expected to contain 100 µg/ml of OFLO and 100 µg/ml of CEFPO. This solution (1.0 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final

concentration of OFLO (10 µg/ml) and CEFPO (10 µg/ml). The responses of the sample solution were measured at 297 nm and 236.2 nm for quantitation of OFLO and CEFPO, respectively. The amounts of the OFLO and CEFPO present in the sample solution were calculated by fitting the responses into the regression equation for OFLO and CEFPO in the proposed method.

## RESULTS AND DISCUSSION

The standard solutions of OFLO and CEFPO were scanned separately in the UV range and zero-order spectra for OFLO (Figure 3) and CEFPO (Figure 4) were recorded. Maximum absorbance was obtained at 297 nm and 236.2 nm for OFLO and CEFPO, respectively. These two wavelengths can be employed for the determination of OFLO and CEFPO without any interference from the other drug in their combined formulations. Overlain zero-order absorption spectrum of OFLO and CEFPO in methanol is shown in Figure 5.

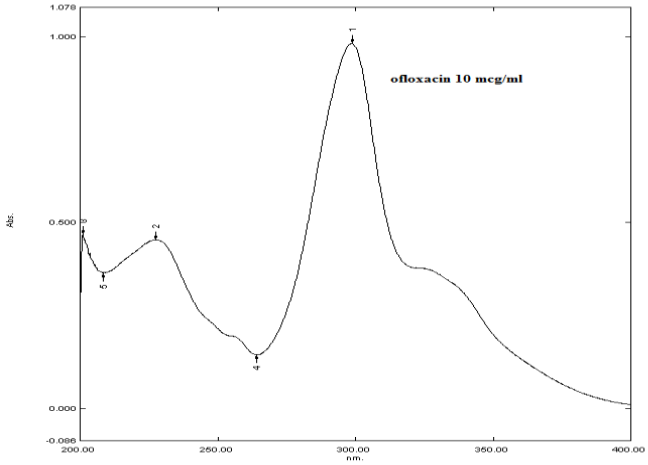


**Figure 3: Zero-order absorption spectra of OFLO in methanol**

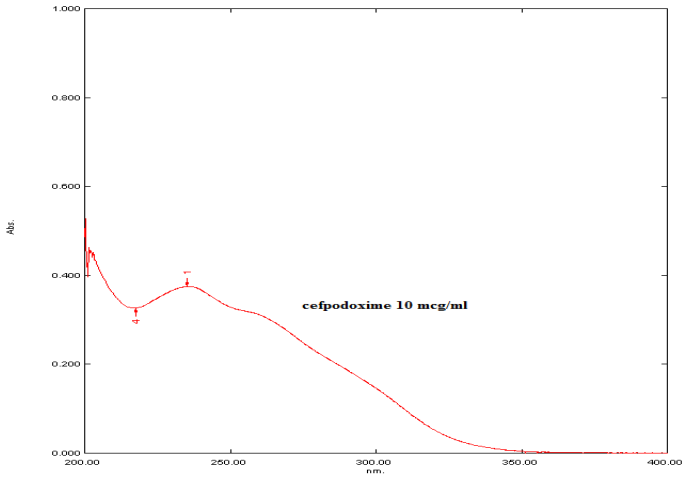


**Figure 4: Zero-order absorption spectra of CEFPO in methanol**

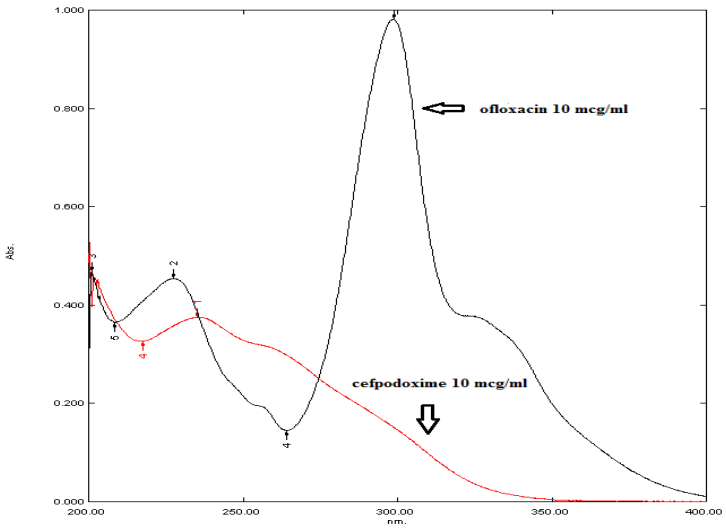


**Figure 5: Overlain zero-order absorption spectra of OFLO and CEFPO in methanol**

Linear correlation was obtained between absorbances and concentrations of OFLO and CEFPO in the concentration ranges of 2-12 µg/ml and 4-24 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values of OFLO were found to be 0.92 and 1.0 % at 297 and 236.2 nm, respectively. The RSD value of CEFPO was found to be 1.09 and 1.19 % at 236.2 and 297 nm, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.38-0.50% and 0.41-0.72% for OFLO at 297 and 236.2 nm, respectively and 0.38-0.64% and 0.44-0.77% for CEFPO at 236.2 and 297 nm, respectively) and intraday (0.11-0.68% and 0.21-0.53% for OFLO at 297 and 236.2 nm, respectively and 0.21-0.51% and 0.30-0.62% for CEFPO at 236.2 and 297 nm, respectively) variation for OFLO and CEFPO, reveal that the proposed method is precise. LOD and LOQ values for OFLO were found to be 0.31 and 0.37 µg/ml and 0.94 and 1.12 µg/ml at 297 and 236.2 nm, respectively. LOD and LOQ values for CEFPO were found to be 0.29 and 0.37 µg/ml and 0.87 and 1.13 µg/ml at 236.2 and 297 nm, respectively. These data show that method is sensitive for the determination of OFLO and CEFPO. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.63 ± 0.47 and 99.57 ± 0.36 for OFLO and CEFPO, respectively (Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine OFLO and CEFPO in their combined dosage form. The results obtained for OFLO and CEFPO were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest

appeared; hence the proposed method is applicable for the routine simultaneous estimation of OFLO and CEFPO in pharmaceutical dosage forms.

**Table 1: Regression Analysis Data and Summary of Validation Parameters for OFLO and CEFPO by Spectrophotometric Method**

| Parameters | OFLO | | CEFPO | |
|---|---|---|---|---|
| Wavelength (nm) | 297 | 236.2 | 236.2 | 297 |
| Beer's law limit (µg /ml) | 2-12 | 2-12 | 4-24 | 4-24 |
| Sandell's sensitivity (µg/cm$^2$/0.001 Absorbance Unit) | 0.0126 | 0.0249 | 0.0281 | 0.0621 |
| Regression equation (y = a + bc) | y = 0.0972x + 0.0366 | y = 0.0404x + 0.0073 | y = 0.0387x - 0.024 | y = 0.016x + 0.003 |
| Slope (b) | 0.0972 | 0.0404 | 0.0387 | 0.016 |
| Intercept (a) | 0.0366 | 0.0073 | -0.024 | 0.003 |
| Correlation coefficient ($r^2$) | 0.9993 | 0.9984 | 0.9997 | 0.9993 |
| LOD[a] (µg/ml) | 0.31 | 0.37 | 0.29 | 0.37 |
| LOQ[b] (µg /ml) | 0.94 | 1.12 | 0.87 | 1.13 |
| Repeatability (% RSD[c], n = 6) | 0.92 | 1.0 | 1.09 | 1.19 |
| Precision (% RSD, n = 3) | | | | |
| Interday | 0.38-0.50 | 0.41-0.72 | 0.38-0.64 | 0.44-0.77 |
| Intraday | 0.11-0.68 | 0.21-0.53 | 0.21-0.51 | 0.30-0.62 |
| Accuracy ± S. D[d]. (% Recovery, n = 5) | 99.63 ± 0.47 | | 99.57 ± 0.36 | |

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

**Table 2: Recovery Data of OFLO and CEFPO by Spectrophotometric Method**

| Drug | Amount taken (µg/ml) | Amount added (%) | % Recovery ± S. D. (n = 5) |
|---|---|---|---|
| OFLO | 4 | 50 | 99.30 ± 0.42 |
| | 4 | 100 | 100.2 ± 0.90 |
| | 4 | 150 | 99.40 ± 0.10 |
| CEFPO | 4 | 50 | 98.10 ± 0.12 |
| | 4 | 100 | 99.20 ± 0.16 |
| | 4 | 150 | 101.4 ± 0.80 |

S. D. = Standard deviation. n = Number of determinations.

**Table 3: Analysis of OFLO and CEFPO by Spectrophotometric Method**

| Tablet | Label claim (mg) | | Amount found (mg) | | % Label claim ± S. D. (n = 6) | |
|---|---|---|---|---|---|---|
| | OFLO | CEFPO | OFLO | CEFPO | OFLO | CEFPO |
| I | 10 | 10 | 10.04 | 9.91 | 100.4 ± 0.87 | 99.12 ± 0.65 |
| II | 10 | 10 | 9.97 | 9.87 | 99.73 ± 1.12 | 98.73 ± 1.37 |

S. D. = Standard deviation. n = Number of determinations.

## CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of OFLO and CEFPO in tablet dosage form. The method utilizes easily

available and cheap solvent for analysis of OFLO and CEFPO hence the method was also economic for estimation of OFLO and CEFPO from tablet dosage form. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of OFLO and CEFPO in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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