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Research Article

# SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETIL AND OFLOXACIN IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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**ABSTRACT**

A new, simple, rapid, accurate, precise and sensitive method has been developed for the simultaneous estimation of Cefpodoxime proxetil and Ofloxacin in their combined dosage form. The method was carried out on a Hiber $C_{18}$ column (250 mm×4.6mm, i.d.5 µm) with a mobile phase consisting of acetonitrile: phosphate buffer pH 3 (pH adjusted with orthophosphoric acid) (75:25) at a flow rate of 1 ml/min and the detection was carried out at 271 nm. The retention time of Cefpodoxime proxetil and Ofloxacin was 3.24 and 2.16 min respectively. Linearity for Cefpodoxime proxetil and Ofloxacin were found in the range of 5-25 µg/ml. The developed method was validated in terms of linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). The proposed method can be used for estimation of both drugs in their combined dosage form.

**Keywords:** Cefpodoxime proxetil, Ofloxacin, RP- HPLC, Validation, Tablet.

## INTRODUCTION

Cefpodoxime proxetil, chemically, [(R, S)-1(isopropoxy carbonyloxy) ethyl (+) - (6R, 7R)-7[2-(2-amino-4-thiazolyl)-2(Z) methoxyiminoacetamido]-3-methoxymethyl-8-oxo-5-thia-1-azabicyclo [4.2.0.]Oct-2-ene-2-carboxylate] is an oral third generation cephalosporin antibiotic. It is active against most gram positive and gram negative bacteria[1]. It is official in IP[2] and USP[3]. IP and USP have described liquid chromatography method for its estimation. HPTLC[4], stability indicating RP-HPLC[5] and RP-HPLC method in plasma[6] for Cefpodoxime proxetil have been reported. RP-HPLC[7] and spectrophotometric[8] methods for simultaneous determination of Cefpodoxime proxetil with other drugs have also been reported.

Ofloxacin, chemically, (R, S)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3,-de]-1,4-benzoazeine-6-carboxylic acid is a fluoroquinolone antibiotic[9]. It is indicated in treatment of respiratory tract, skin, soft tissue, urinary tract infection and gonorrhea[10]. It is official in IP[9], BP[11] and USP[12]. Potentiometric method has been described in IP, BP and USP for its estimation. For Ofloxacin, flow injection spectrophotometry[13], HPLC with fluorescence detector[14], chemiluminescence method[15], LC-MS/MS method[16] and differential pulse polarographic methods[17] have been reported. UV[18], RP-HPLC[19-20], stability indicating RP- HPLC[21], HPTLC[22] methods for simultaneous estimation of Ofloxacin with other drugs have also been reported.

Cefpodoxime proxetil and Ofloxacin in combined dosage form is approved on 16-4-2011 by Central Drug Standard Control Organization (CDSCO) and till now, no chromatographic method for simultaneous estimation of Cefpodoxime proxetil and Ofloxacin in combined dosage form is reported. Hence an attempt has been made to develop a new RP-HPLC method for their simultaneous estimation in pharmaceutical dosage form.

## MATERIAL AND METHOD

### Chemicals and reagents

Analytical pure Cefpodoxime proxetil and Ofloxacin were obtained as a gift sample from Nirlife Healthcare Ltd., Sachana, Ahmedabad, Gujarat, India. The formulation Cepodem®-O tablet (Akems Drugs &Pharmaceuticals Ltd, Ranipur, Haridwar, India) was procured from the local market with labeled amount of 200 mg of Cefpodoxime proxetil and 200 mg of Ofloxacin. Acetonitrile, methanol and water were used of HPLC grade, purchased from RANKEM Ltd. Ortho phosphoric acid was of analytical grade purchased from RANKEM Ltd. Disodium hydrogen phosphate and citric acid were purchased from ASTRON CHEMICALS Ltd.

### Instrumentation and chromatographic condition

The LC system (YL-9100) consisted of following components: YL9160 PDA detector, YL9101 vacuum degasser and YL9110 quaternary solvent delivery pump. Chromatographic analysis was carried out on a Hiber $C_{18}$ column (250 mm×4.6mm, i.d.5 µm) using mobile phase acetonitrile: phosphate buffer pH-3 (pH adjusted with orthophosphoric acid) 75:25 with flow rate of 1ml/min. Detection of eluent was made at 271 nm by PDA detector. The column was maintained at room temperature and injection volume of 20µl was used. The mobile phase was filtered through 0.45µm Chrom Tech Nylon-66 filter paper.

### Preparation of standard solution

Standard stock solution of pure drugs were prepared separately by dissolving 12.5 mg of each drug with mobile phase in 25 ml of volumetric flask and made up to volume to get concentration of 500 µg/ml. 1 ml from stock solution of Cefpodoxime proxetil and 1 ml from stock solution of Ofloxacin were mixed in 10 ml of volumetric flask and made up to volume with mobile phase to get a mixed standard solution containing 50 µg/ml of Cefpodoxime proxetil and Ofloxacin both.

### Preparation of sample solution

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 25 mg of Ofloxacin was transferred to 50 ml volumetric flask containing 40 ml of mobile phase, gentle shaking was carried out for 5min and ultrasonicated for 5 min. The volume was made up to the mark with the mobile phase. The tablet sample solution was filtered through Whatman filter paper no.41. 1 ml of filtrate was further diluted to 10 ml of mobile phase to get 50 µg/ml concentrations. From the above solution 2 ml was further diluted to 10 ml with mobile phase to get the final concentration 10 µg/ml. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present in tablet was estimated from their respective calibration curve (Table-1).

### System suitability

The system suitability was assessed by six replicate injections of the mixture containing 25µg/ml of both the drugs. The resolution, peak asymmetry and number of theoretical plates were calculated (Table 2). The obtained values were demonstrated the suitability of the system for the analysis of these drugs in combination.

**Method validation**

The method was validated for linearity, accuracy, intraday and interday precision, LOD and LOQ, in accordance with ICH guideline [23].

**Linearity**

Aliquots 1,2,3,4 and 5 ml of mixed standard solution of Cefpodoxime proxetil and Ofloxacin were transferred to series of 10 ml volumetric flasks and made up to volume with mobile phase. Each solution was injected and chromatogram was recorded. Retention time (mean ± s.d[a]) of Cefpodoxime proxetil and Ofloxacin were found to be 3.248 ± 0.003 and 2.166 ± 0.003 min respectively. The peak area of Cefpodoxime proxetil and Ofloxacin in each chromatogram was recorded.

s.d[a]= standard deviation.

**Accuracy**

To study accuracy of the method, recovery studies were carried out by addition of standard drug sample in a tablet sample at 50%, 100% and 150%. The percentage of recovery was calculated (Table-3).

**Precision**

It was carried out by preparing 3 replicates of 3 different concentrations within the linearity range and then injecting each solution. The peak area of Cefpodoxime proxetil and Ofloxacin in each chromatogram was recorded in order to record any intra day variation. To record inter day variation, 3 different concentration solution within the linearity range were analyzed for 3 different days. The peak area of each drug was recorded and % RSD (% relative standard deviation) was calculated for both series of analysis.

**Limit of detection (LOD) and limit of quantification (LOQ)**

They were calculated as 3.3 σ/S and 10 σ/S respectively. Where σ is the standard deviation of the response (y- intercept) and S is the mean of the slope of calibration plot.

**RESULTS AND DISCUSSION**

For RP-HPLC method, several different mobile phases were tried and finally mobile phase containing acetonitrile: phosphate buffer pH-3 (pH adjusted with orthophosphoric acid) 75:25 was found to be optimized and well defined. Resolved peaks of Cefpodoxime proxetil and Ofloxacin with retention time (mean ± s.d.) of 3.248 ± 0.003 and 2.166 ± 0.003 min were obtained respectively. The representative chromatogram of mixed standard solution of Cefpodoxime proxetil and Ofloxacin (25 µg/ml) is shown in Fig 1 and 3D view of different concentrations of mixed standard solutions of Cefpodoxime proxetil and Ofloxacin is shown in Fig 2. The calibration curve for each drug was obtained separately by plotting as peak area → concentration over the range of 5-25 µg/ml. From, calibration curve of Cefpodoxime proxetil (Fig 3), it was found to linear with $r^2$= 0.9985 and from calibration curve of Ofloxacin (Fig 4) it was found to linear with $r^2$= 0.9981. The % recoveries for Cefpodoxime proxetil and Ofloxacin were found to be 99.06-100.8 and 99.26-101.95 respectively, which were satisfactory (Table-3). The precision is usually expressed as % RSD. The intraday precision for Cefpodoxime proxetil and Ofloxacin were found to be 0.4018-0.1279 and 0.1042-0.2103 respectively. The inter day precision for Cefpodoxime proxetil and Ofloxacin were found to be 0.4000-1.2159 and 0.8831-1.4496 respectively. The limit of detection (LOD) for Cefpodoxime proxetil and Ofloxacin were 0.2698 and 1.2500 µg/ml respectively. The limit of quantification (LOQ) for Cefpodoxime proxetil and Ofloxacin were 0.8178 and 3.8100 respectively. The system suitability parameters for RP- HPLC are shown in Table-2.

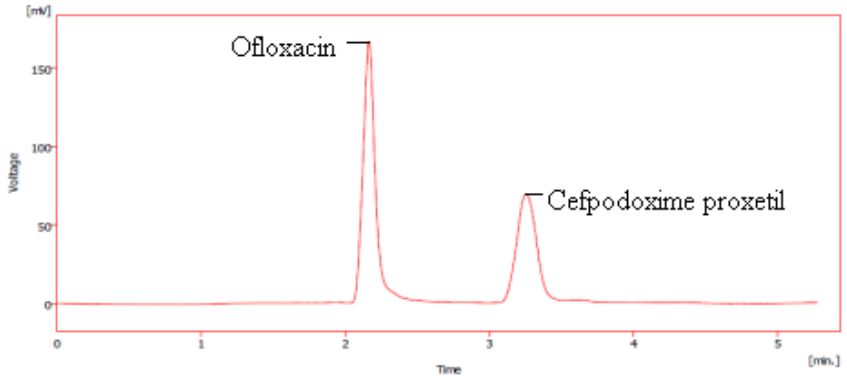


**Fig. 1: Representative chromatogram obtained for mixed standard solution of Cefpodoxime proxetil (25 µg /ml, 3.248 ± 0.003 min) and Ofloxacin (25 µg/ml, 2.166 ± 0.003 min)**

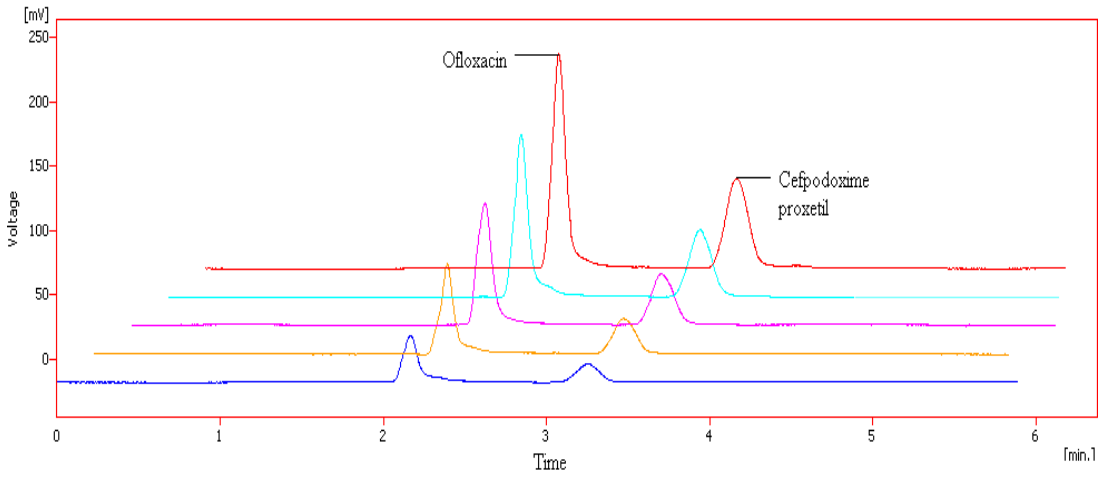


**Fig. 2: 3D view of different concentrations of mixed standard solutions of Cefpodoxime proxetil (3.248 ± 0.003 min) and Ofloxacin (2.166 ± 0.003 min)**

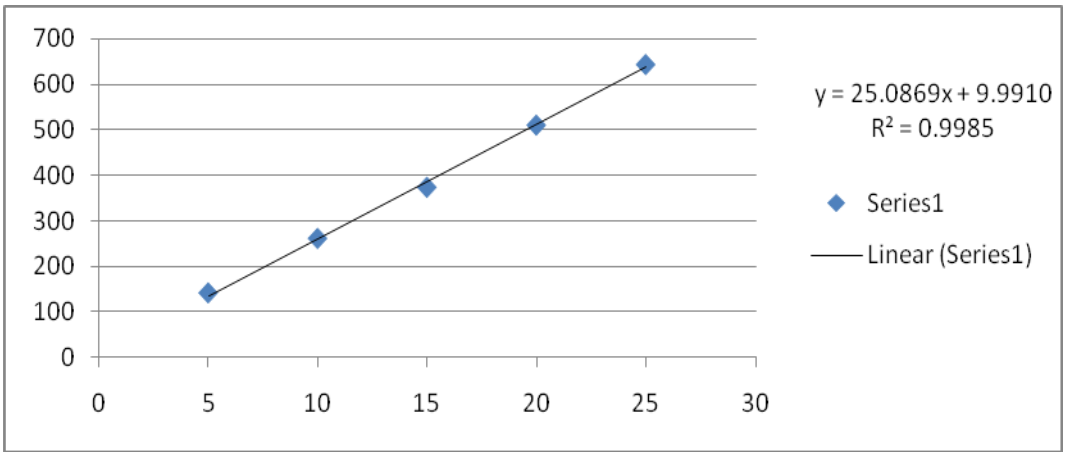


**Fig. 3: Calibration curve of Cefpodoxime proxetil**

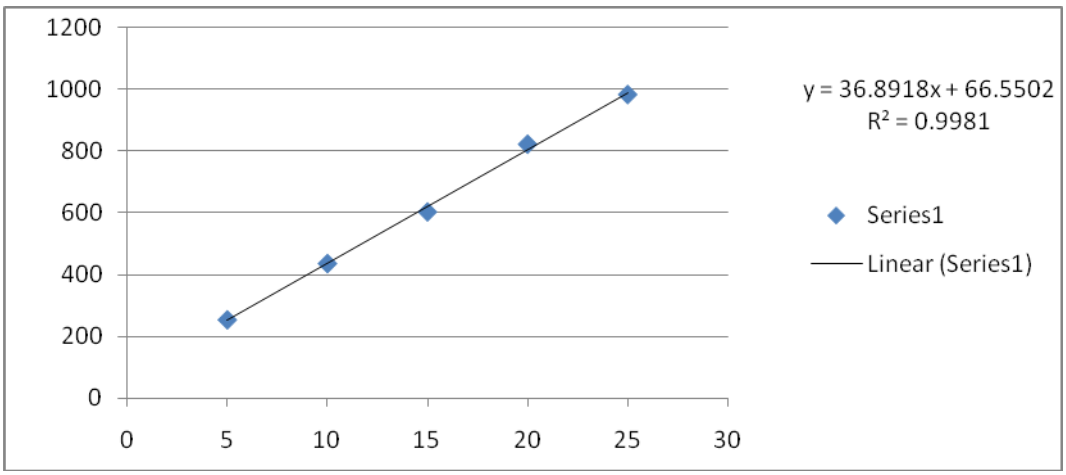


**Fig. 4: Calibration curve of Ofloxacin**

**Table 1: Assay results of combined dosage form**

| Drug | Labeled claim (mg) | Amount found (mg) * | % label claim |
|---|---|---|---|
| Cefpodoxime proxetil | 200 | 200.4 | 100.2 |
| Ofloxacin | 200 | 200.4 | 100.2 |

*Each value is a mean of six observations.

**Table 2: System suitability parameters for RP-HPLC**

| S. no. | Parameters | Cefpodoxime proxetil * | Ofloxacin* |
|---|---|---|---|
| 1 | No. of theoretical plates | 2489 | 3201 |
| 2 | Asymmetry factor | 1.162 | 1.120 |
| 3 | Tailing factor | 1.160 | 1.241 |
| 4 | Resolution | 5.270 | - |

*Each value is a mean of six observations.

**Table 3: Recovery studies of Cefpodoxime proxetil and Ofloxacin**

| Drug | Amount taken(µg/ml) | Amount added (µg/ml) | Total amount found (µg/ml) | % recovery |
|---|---|---|---|---|
| Cefpodoxime proxetil | 10 | 5 | 14.86 | 99.06 |
| | 10 | 10 | 19.97 | 99.85 |
| | 10 | 15 | 25.2 | 100.8 |
| Ofloxacin | 10 | 5 | 14.89 | 99.26 |
| | 10 | 10 | 20.39 | 101.95 |
| | 10 | 15 | 24.85 | 99.4 |

**Table 4: Summary of validation parameters of proposed RP-HPLC**

| Parameters | Cefpodoxime proxetil | Ofloxacin |
|---|---|---|
| Linearity (µg/ml) | 5-25 | 5-25 |
| Correlation coefficient | 0.9985 | 0.9981 |
| Slope (m) | 25.086 | 36.890 |
| Intercept (c) | 9.991 | 66.55 |
| LOD [b] (µg/ml) | 0.2698 | 1.2500 |
| LOQ [c] (µg/ml) | 0.8178 | 3.8100 |
| Accuracy(% recovery) | 99.06-100.8 | 99.26-101.95 |
| Precision (%RSD) [d] | | |
| Intraday ($n^e$=9) | 0.1279-0.4018 | 0.1042-0.2103 |
| Inter day ($n^e$=9) | 0.4000-1.2159 | 0.8831-1.4496 |

LOD[b]= limit of detection; LOQ[c]=limit of quantification; (%RSD)[d]= % relative standard deviation, $n^e$ = number of observations.

## CONCLUSION

The validated RP-HPLC method employed here is simple, rapid, accurate, precise, sensitive and cost effective which can be used for routine analysis of Cefpodoxime proxetil and Ofloxacin in combined pharmaceutical dosage form.

## ACKNOWLEDGEMENT

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