Journal of Applied Pharmaceutical Science Vol. 4 (05), pp. 046-050, May, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.40508
ISSN 2231-3354

Simultaneous estimation of Cefpodoxime proxetil and Ofloxacin In tablet dosage form using RP-HPLC

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ARTICLE INFO

Article history:
Received on: 17/03/2014
Revised on: 20/04/2014
Accepted on: 06/05/2014
Available online: 27/05/2014

Key words:
Cefpodoxime proxetil, Ofloxacin, RP-HPLC method development and Validation.

ABSTRACT

A simple, rapid and precise reverse phase liquid chromatographic (RP-HPLC) method was developed and subsequently validated for simultaneous estimation of Cefpodoxime proxetil and Ofloxacin in combined fixed dose oral formulation. The analysis was carried out using X-terra C8 (4.6 x 250mm, 5µm, Make: ACE), pre-packed column. The separation was carried out using a mobile phase containing a 0.25%v/v triethyl amine buffer of pH 3.5 and acetonitrile (30:70 v/v), was pumped at a flow rate of 1.2 ml/min with UV-detector and PDA detection at 227 nm. Both the drugs were well resolved on the stationary phase and the retention times were around 2.747 minute for Cefpodoxime proxetil and 2.076 minute for Ofloxacin. The method was validated and shown to be linear for Cefpodoxime proxetil and Ofloxacin. The correlation coefficients for Cefpodoxime proxetil and Ofloxacin are 0.998 and 0.999 respectively. The relative standard deviations for five replicate measurements in two sets of each drug in the tablets is always less than 2% and mean % error of active recovery not more than ±1.5%. The method was validated for precision and accuracy. The developed method could be applied for routine analysis of Cefpodoxime proxetil and Ofloxacin in tablet dosage form without any interference of excipients.

INTRODUCTION

Cefpodoxime proxetilis (CPD), [(R, S)-1(isopropoxycarbonyloxy) ethyl (+) - (6R, 7R)-7[2-(2-amino-4-thiazolyl)-2(Z)methoxyiminoacetamido]-3-methoxymethyl-8-oxo-5-thia-1azabicyclo [4.2.0.] Oct-2-ene-2-carboxylate] **(Fig 1)** is an orally administered, extended spectrum, semi-synthetic, third generation oral cephalosporin. It is a pro-drug of Cefpodoxime and is indicated for the treatment of patients with mild to moderate infections like Pharyngitis and/ or tonsillitis, Community-acquired pneumonia, Acute bacterial exacerbation of chronic bronchitis, Acute uncomplicated urethral and cervical gonorrhea, Acute uncomplicated ano-rectal infections in women, Uncomplicated skin and skin structure infections, Acute maxillary sinusitis and Uncomplicated urinary tract infections (cystitis) (John & John, 2004; Borin, 1991; Bergogne, 1991; Geddes, 1991; Kakumanu, 2006; Chocas, 1993). CPD is the subject of a monograph in the Indian Pharmacopoeia and United States Pharmacopoeia (Indian Pharmacopeia, 2010; United Pharmacopeia, 2005). Ofloxacin (OFL), (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-carboxylic acid is a fluoroquinolone antibacterial **(Fig 2)**, used in the treatment of chalmydia or chalmydophilia infections including nongonococcal urethritis, mycobacterial infections such as leprosy, Acute bacterial exacerbation of chronic bronchitis, Community-acquired pneumonia, Uncomplicated skin infections, Non-gonococcal cervicitis/ urethritis due to Chlamydia trachomatis and *Neisseria gonorrhea*, Pelvic inflammatory disease, Uncomplicated cystitis, Complicated urinary tract infections, Chronic bacterial prostatitis, Traveller's diarrhoea, Typoid fever and Legonnaire's disease (John H, John M, 2004). OFL is the subject of the Indian Pharmacopoeia, United States Pharmacopeia and British Pharmacopeia (Indian Pharmacopoeia, 2010; British Pharmacopoeia, 2010; United State Pharmacopoeia).

Cefpodoxime proxetil and Ofloxacin are formulated together in the form of tablet. Literature reveals that potentiometric, spectrofluorimetric, chromatographic methods have been reported for their individual analysis, along with other combinations in

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pharmaceutical formulation and biological fluids (Camus et al., 1994; Malathi et al., 2009; Lovdahl et al., 1994; Kakumanu et al., 2006; Patel et al., 2011; Molina et al., 1991; Stoeckel et al., 1998; Fukutsu et al., 2006; Jain et al., 2012; Wang et al., 2007; Garcia et al., 2005; Wongsinsup et al., 2009; Rao et al., 2000; Meredith et al., 2012; Rizk et al., 1998). However, most of these methods are uneconomic and environmentally unfriendly because of complex sample preparation, high solvent consumption along with long analytical run time made these procedures unsuitable for routine analysis (Khandagle et al., 2011; Karanam et al., 2012; Darshan et al., 2012). Moreover, when we tried to follow the proposed method (Sandeep et al., 2012) we did not achieve good resolution and method requires buffer in mobile phase and has longer retention time. Hence, the aim of the present investigation was to develop and validate a economic, simple, feasible, rapid, sensitive, and specific RP-HPLC method for the quality control of a Cefpodoxime proxetil and Ofloxacin in pharmaceutical preparations with lower solvent consumption along with the short analytical run time leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. The proposed method is applicable as well as for routine analysis and content uniformity test of Cefpodoxime proxetil and Ofloxacin in tablets and complies well with the validation requirements in the pharmaceutical industry.

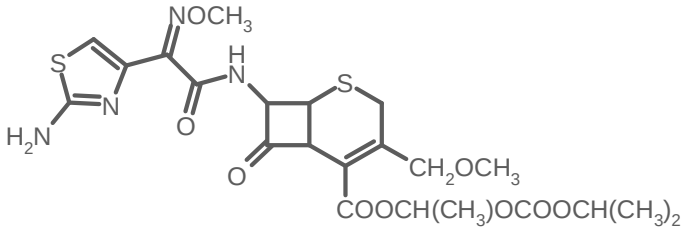

Fig. 1: Chemical structure of Cefpodoxime proxetil.

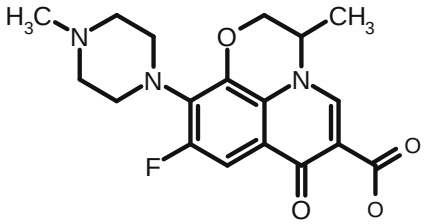

Fig. 2: Chemical structure of Ofloxacin.

EXPERIMENT

Equipment and chromatographic conditions

A high-performance liquid chromatographic system (waters, software: EMPOWER) equipped with auto sampler and DAD or UV detector. All pH measurements were performed on a pH meter (Sentron, Netherlands).Chromatographic separation was carried out at room temperature with X terra C8 (4.6 x 250mm, 5µm, Make: ACE) column. For the mobile phase, 2.5 ml of Triethylammonium was dissolved in 900 ml of double-distilled water. The pH of the Triethyl amine was adjusted to 4.5 ± 0.05 with orthophosphoric acid.

The buffer solution was shaked manually to mix and finally make the volume up to 1000 ml with the water. A mixture of Triethyl amine and acetonitrile inthe ratio of 30: 70 was prepared. Finally the mobile phase was filtered through a 0.45 µm membrane filter and degassed for 10 minutes. The injection volumes for samples and standards were 20 µl and eluted at a flow rate of 1.2ml/min at 40 °C. The eluents were monitored at 227 nm.

Reagents and chemicals

Acetonitrile and methanol were of HPLC grade and werepurchased from E. Merck, Darmstadt, Germany.Triethyl amine, orthophosphoric acid and other reagents were of analytical-reagent grade and purchased from E.Merck, Darmstadt, Germany. Water was deionised and double distilled. Cefpodoxime proxetil and Ofloxacin bulk powder was obtained from Esteem laboratories, Hyderabad. The marketed preparation of the given combination is procured from local market.

Preparation of standard solutions

A working standard solution containing Cefpodoxime Proxetil and ofloxacin was prepared by weighing 10 mg of Cefpodoxime proxetil and Ofloxacin dissolve in 100 ml mobile phase. The mixture was sonicated for 5minutes or until the reference standard dissolved completely. 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 10 µg/ml of Cefpodoxime proxetil and Ofloxacin both.

Preparation of sample solutions

Twenty tablets, each containing 200 mg Cefpodoxime proxetil and 200 mg Ofloxacin were accurately weighed and finely powdered. A quantity of powder equivalent to 10 mg of Cefpodoxime Proxetil and Ofloxacin was weighed and transferred to a 100 ml volumetric flask. About 70 ml ofmobile phase was added and shaked mechanically for 15 minutes. The mixture was then sonicated in ultrasonic bath for 5 minutes and makes the volume up to 100 ml by the mobile phase. 1 ml from above solution is taken and diluted to 10 ml using mobile phase.The solution was filtered with a Whatman filter paper no.1. Before injection, both standard and sample solutions were filtered through 0.45 µm syringe filter. Then 20 µl of standard and sample solutions were injected into column and chromatogram was recorded.

Method validation

Linearity

In order to check the linearity for the developed method, solutions of five different concentrations ranging from 5-25 µg / ml were prepared for CPD and 5-25 µg / ml for OFL, respectively.

The Chromatograms peak areas were recorded and calibration curve was plotted of peak area against concentration of drug. The chromatograms were recorded and the peak areas are given in Table 1.

A linear relationship between areas versus concentrations was observed in the above-mentioned linearity range. This range was selected as the linear range for the development of the analytical method, for the estimation of CPD and OFL. The calibration curves for both drugs given in Fig 3 and Fig 4.

Table. 1: Linearity data for Cefpodoxime proxetil and Ofloxacin.

CPD conc. (µg / ml)	Mean peak area of CPD	OFLconc. (µg / ml)	Mean peak area of OFL
5	1086612	5	980166
10	2176885	10	1907017
15	3092395	15	2992597
20	4152668	20	3779408
25	5109975	25	4655870

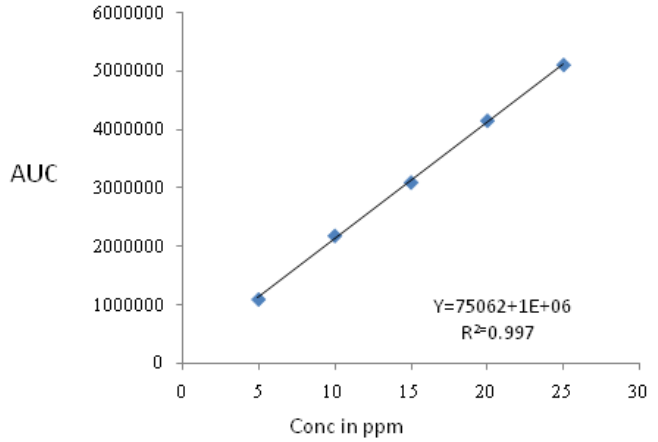

Fig. 3: Calibration curve of Cefpodoxime proxetil at 227 nm.

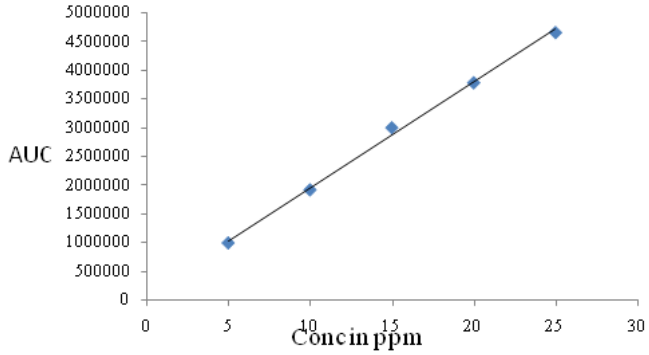

Fig. 4: Calibration curve of Ofloxacin at 227 nm.

Sensitivity

The sensitivity of the measurement of CPD and OFL using the proposed method was estimated as the limit of quantification (LOQ) and the lowest concentration detected under these chromatographic conditions as the limit of detection (LOD). The LOD and LOQ were calculated by using the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S, where σ was the standard deviation of the peak areas of the drug (n = 5), and S was the slope of the corresponding calibration plot. The limits of detection and quantification for CPD were 0.033µg / ml and 0.010 µg / ml, respectively, and those for OFL were 0.004 µg / ml and 0.013 µg / ml, respectively.

System suitability

Various system suitability parameters were also calculated. It was observed that all the values were within the limits, and is shown in Table 2. The statistical evaluation of the proposed method revealed its good linearity, reproducibility, and its validation of different parameters and led us to the conclusion that it could be used for the rapid and reliable determination of CPD and OFL in tablet formulation. The results are furnished in Table 2.

Table 2: System suitability parameters for Cefpodoxime proxetil and Ofloxacin.

Parameter (*n = 5)	CPD	OFL
Retention time	2.747	2.076
Plate Count	2606.38	2531.76
USP Resolution	2.99	2.97
USP Tailing	1.67	1.44

* Five replicates, CPD- Cefpodoxime proxetil; OFL- Ofloxacin

Precision

Precision was measured by the analysis of sample solutions three times at three different concentrations. Solutions containing 10, 15, and 20 µg / ml of CPD and 10, 15, and 20 µg / ml of OFL were subjected to the proposed HPLC analysis, to check the intraday and inter day variations of the method. The results are furnished in **Tables 3** and **4**.

Table. 3: Results of the intraday precision.

Cefpodoxime proxetil			Ofloxacin		
Conc. (µg / ml)	Peak area Mean S.D (n = 3)	RSD (%)	Conc. (µg / ml)	Peak area Mean S.D (n = 3)	RSD (%)
10	8215.3	0.35	10	7154.1	0.37
15	8634.9	0.31	15	7752.1	0.33
20	8573.2	0.38	20	7943.9	0.35

Table. 4: Results of the interday precision.

Cefpodoxime proxetil			Ofloxacin		
Conc. (µg / ml)	Peak area Mean S.D (n = 3)	RSD (%)	Conc. (µg / ml)	Peak area Mean S.D (n = 3)	RSD (%)
10	8055.8	0.25	10	7832.5	0.43
15	7858.3	0.31	15	7152.6	0.45
20	8397.4	0.33	20	8041.5	0.38

Accuracy

The accuracy of the method was determined by the analysis of standard additions at three levels, that is, multiple-level recovery studies. The reference standard, at three different concentrations (50, 100, and 150 %), was added to a fixed amount of the pre analyzed sample and the amounts of the drug were analyzed by the proposed method. Results from the recovery studies are given in **Tables 5** and **6**.

Table. 5: Results of the recovery study of Cefpodoxime proxetil.

Amt of CPD in sample (µg)	Amt. of Std. CPD added (µg)	Total amt. of CPD (µg)	Total amt. of CPD found (µg) Mean ± S.D.	Total amt recovered (µg)	% Recovery (n = 3)
10	05	15	14.95	14.95	99.05%
10	10	20	19.96	19.96	99.56%
10	15	25	24.74	24.74	98.29%

Table. 6: Results of the recovery study of Ofloxacin.

Amt of OFL in sample (µg)	Amt. of Std. OFL added (µg)	Total amt. of OFL (µg)	Total amt. of OFL found (µg) Mean ± S.D.	Total amt recovered (µg)	% Recovery (n = 3)
10	05	15	15.07	15.07	101.36%
10	10	20	19.99	19.99	99.87%
10	15	25	24.76	24.76	98.39%

Solution stability

The stability of CPD and OFL standard and sample solutions was determined by storing the solutions at an ambient temperature (20 ± 10°C). The solutions were checked in triplicate after three successive days of storage and the data were compared with the freshly prepared samples. In each case, it could be noticed that the solutions were stable for 48 hours, as during this time the results did not decrease below 98%. This showed that CPD and OFL were stable in standard and sample solutions for at least two days, at ambient temperature.

Robustness

The robustness of the method was determined by making slight changes in the chromatographic conditions like flow rate (±0.1), temperature (±5), and pH (±0.2) of the mobile phase. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed was robust.

RESULTS AND DISCUSSION

The RP-HPLC procedure was optimized with a view to develop an accurate and stable assay method with the pure drugs CPD and OFL, in a tablet formulation. X terra C8 (4.6 x 250mm, 5µm, Make: ACE) column in isocratic mode was used, with a mobile phase of 2.5 ml of Triethylammonium was dissolved in 900 ml of double-distilled water. The pH of the Triethyl amine was adjusted to 4.5 ± 0.05 with orthophosphoric acid. The flow rate was 1.2 ml/min at 40 °C and identical components were measured, with detection at 227 nm. Linearity was assessed by plotting concentration versus area, which is shown in Table 1, and it is linear in the range of 5 – 25 µg / ml for CPD and 5 – 25 µg / ml for OFL, with correlation coefficients of 0.9998 and 0.9995, respectively, with a good linearity response, greater than 0.999. The % recovery was found to be within limits of the acceptance criteria with a recovery range of 99.05% – 99.56% % for CPD and 98.39% – 101.36% for OFL. The %RSD for intraday and Interday precision was less than 2% for CPD and OFL. The detection limit of the proposed method was 0.033 µg/ml and 0.004 µg/ml, and the quantification limit was 0.010 µg/ml and 0.013 µg/ml for CPD and OFL, respectively. A typical chromatogram of the standard solution of CPD and OFL at the test level is shown in **Fig 5,** and a chromatogram of the test solution is shown in **Fig 6**. The assay procedures were repeated six times and the results were found to give 99.33 % of CPD and 99.98% of OFL as shown in **Table 7**.

Table. 7: Results of the analysis of the test preparation.

Formulation	(Mean ± % R.S.D.)	
	CPD	OFL
%Conc. estimated*	99.33	99.98

* Average of six determinations; R.S.D.: Relative standard deviation CPD- Cefpodxime proxetil; OFL- Ofloxacin.

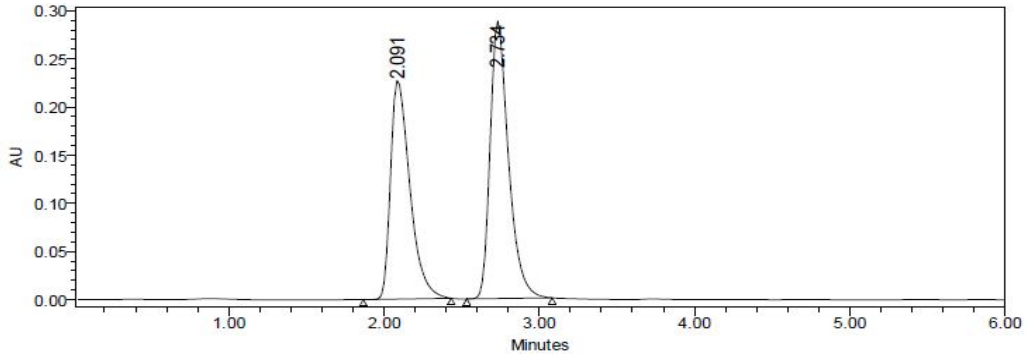

Fig. 5: Chromatogram of the standard preparation of Cepodoxime proxetil and Ofloxacin.

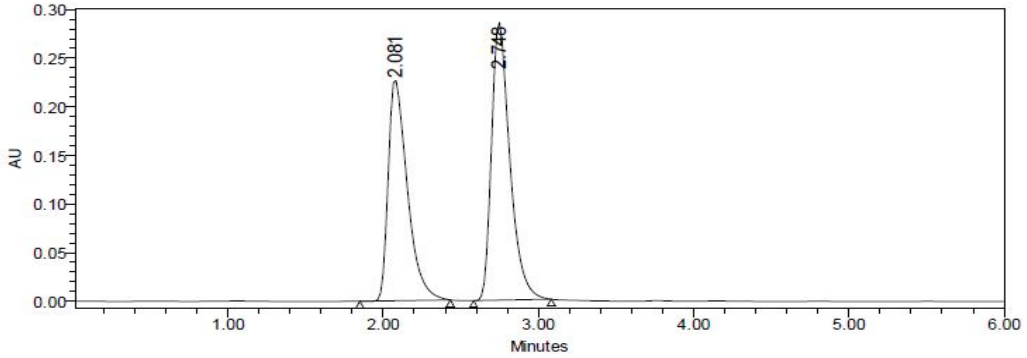

Fig. 6: Chromatogram of the test preparation of Cepodoxime proxetil and Ofloxacin.

CONCLUSIONS

The proposed study describes a new and simple RP-HPLC method for the estimation of CPD and OFL in tablet formulation. The method has been validated and found to be simple, rapid, sensitive, accurate, and precise. Moreover, the lower solvent consumption along with the short analytical run time of 6.0 minutes leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. Therefore, the proposed method can be used for quantification of CPD and OFL in solid oral formulations as well as routine analysis, in quality control.

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How to cite this article:

Annadi Chiranjeevi, Medidi Srinivas., Simultaneous estimation of Cefpodoxime proxetil and Ofloxacin In tablet dosage form using RP-HPLC. J App Pharm Sci, 2014; 4 (05): 046-050.