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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEPPODOXIME PROXETIL AND OFLOXACIN IN TABLET DOSAGE FORM

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ABSTRACT

A simple, selective and rapid reversed phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed and validated for the simultaneous analysis of cefpodoxime proxetil and ofloxacin in tablet dosage form. The separation was carried out using a mobile phase consisting of 20mM phosphate buffer, Acetonitrile and methanol with pH 3.0 adjusted with ortho phosphoric acid in the ratio of 30: 60: 10 % v/v/v. The column used was Thermo C18, (250 mm x 4.6 mm i.d, 5µm) with flow rate of 1 ml / min using PDA detection at 236 nm. The described method was linear over a concentration range of 1-20 µg/ml and 1-20 µg/ml for the assay of cefpodoxime proxetil and ofloxacin respectively. The retention times of cefpodoxime proxetil and ofloxacin were found to be 2.65 and 4.17 min respectively. Results of analysis were validated statistically and by recovery studies. The mean recovery was 99.81 ± 1.18 and 99.87 ± 0.27 for cefpodoxime proxetil and ofloxacin, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) for cefpodoxime proxetil and ofloxacin were found to be 0.17 and 0.19 µg/ml and 0.51 and 0.58 µg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of cefpodoxime proxetil and ofloxacin in its pharmaceutical dosage form.

INTRODUCTION

Ofloxacin (OFLO) is chemically 9-Fluoro-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], is a fluoroquinolone antibacterial, used in the treatment of chlamydia or chlamydomydia 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 spectrophotometric⁶⁻⁷, HPLC⁸⁻⁹ and chemiluminescence^[10] methods for determination of OFLO in pharmaceutical dosage forms as well as in biological fluids. Literature survey also reveals spectrophotometric^[11], RP-HPLC^[12] and HPTLC^[12] methods for determination of OFLO with other drugs. Cefpodoxime proxetil (CEFO) 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], is a third generation cephalosporin antibiotic. It is used for infections of the respiratory tract, urinary tract and skin and soft tissues. It has greater activity against staphylococcus aureus^[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 CEFO. Literature survey also reveals RP-HPLC^[18] and spectrophotometric^[19] methods for determination of CEFO with other drugs. The combined dosage forms of OFLO and CEFO 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 CEFO in their combined dosage forms. The present communication describes simple, sensitive, rapid, accurate and economical chromatographic method (RP-HPLC) for simultaneous estimation of both drugs in their combined tablet dosage forms.

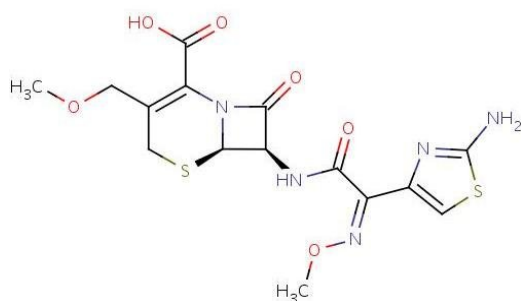
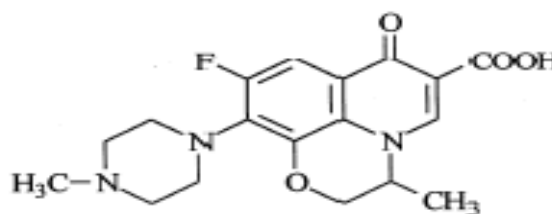


Figure 1: Structural formula of CEFO



Ofloxacin (Oflox)

Figure 2: Structural formula of OFLO

MATERIALS AND METHODS

Apparatus

RP-HPLC instrument (Shimadzu, LC-2010C_{HT}, Japan) equipped with a UV-Visible detector and a photodiode array detector, auto sampler, Thermo C₁₈ column (250 mm × 4.6 mm i.d., 5 µm particle size) was used. Chromatograms were automatically obtained by LC-solution 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

CEFO and OFLO bulk powder was kindly gifted by Acme Pharmaceuticals Ltd. Ahmedabad, India. The commercial fixed dose combination product was procured from the local market. Methanol AR Grade was procured from Finar Chemicals Ltd., Ahmedabad, India.

Preparation of standard stock solutions

A mixed standard solution of CEFO (100 µg/ml) and OFLO (100 µg/ml) was prepared by accurately weighing CEFO (10 mg) and OFLO (10 mg) and dissolving in methanol and diluted to 100 ml with methanol in the same volumetric flask.

Preparation of sample solution

Twenty tablets were weighed and powdered. The quantity of the tablet powder equivalent to 10 mg of both drugs transferred to 100 ml volumetric flasks. The content were mixed with methanol (70 ml) and sonicated for 20 min to dissolve the drug as completely as possible. The solutions were then filtered through a whatman filter paper no. 41 and a volume was adjusted up to the mark with methanol. From this 0.5 ml was transferred to a 10 ml volumetric flask and the volume was adjusted up to the mark with methanol to obtain required concentration of CEFO (5 µg/ml) and OFLO (5 µg/ml).

Preparation of pH 3.0 buffer solution

Potassium di hydrogen phosphate (20 mM, 2.72 gm) in 1000ml of milliequivalent water was solubilised and adjusted the pH to 3.0 ±0.05 with ortho phosphoric acid solution.

Methodology

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for CEFO and OFLO was obtained with a mobile phase Acetonitrile: Methanol: Phosphate buffer (20 mM, PH 3) [60:10:30 v/v/v] at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was carried out at 236 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Figure 3). System suitability test parameters for CEFO and OFLO for the proposed method are reported in Table 1.

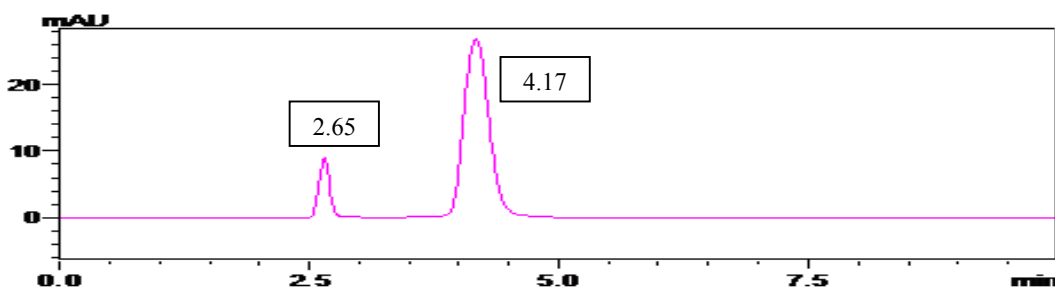


Figure 3: Chromatogram of standard solution of CEFO and OFLO (5 µg/ml) at 236 nm

Table 1: System suitability parameters of chromatogram for

Parameters	CEFO \pm RSD(n = 6)	OFLO \pm RSD(n = 6)
Retention time (min)	2.65 \pm 0.38	4.17 \pm 0.28
Tailing factor	1.062 \pm 0.65	1.161 \pm 0.73
Theoretical plates	2574 \pm 1.00	3328 \pm 0.78
Resolution	3.91 \pm 0.56	

Validation of the proposed method

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

Calibration curve (Linearity)

Calibration curves were constructed by plotting Peak areas vs. Concentrations of CEFO and OFLO, and the regression equations were calculated. The calibration curves were plotted over the concentration range 1-20 µg/ml for CEFO and OFLO. Accurately measured standard working solutions of CEFO and OFLO (0.1, 0.2, 0.5, 0.8, 1.0, 1.2, 1.5 and 2.0) were transferred to a series of 10ml of volumetric flasks and diluted to the mark with Methanol. Aliquots (20 µL) of each solution were injected under the operating chromatographic conditions described above.

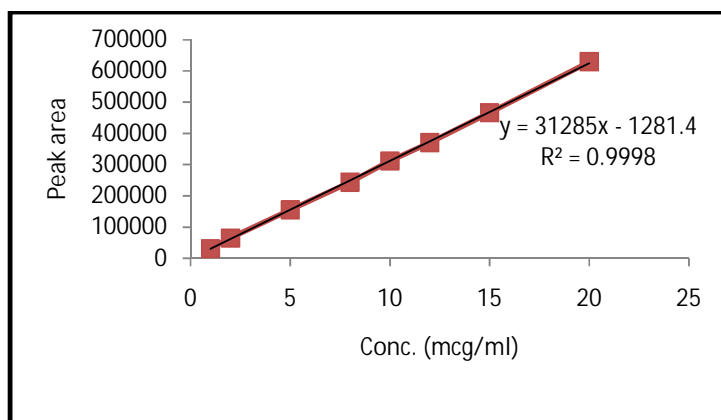


Figure 4: Calibration curve of CEFO at 236 nm

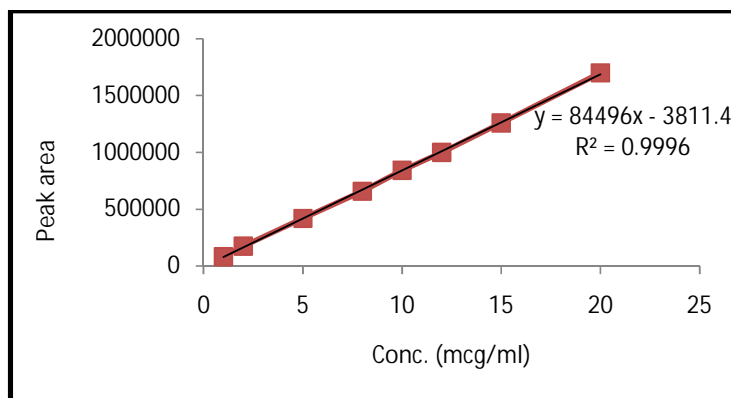


Figure 5: Calibration curve of OFLO at 236 nm

Method precision (Repeatability)

The %RSD values for CEFO and OFLO were found to be 0.215 % and 0.625% respectively. The low %RSD values (<2%) indicates that proposed method is repeatable.

Intermediate precision (Reproducibility)

The low RSD values of interday (0.25-1.10 % and 0.14 – 1.33 %) and intraday (0.11-0.50 % and 0.32 – 1.24 %) for CEFO and OFLO, respectively, reveal that the proposed method is precise.

Limit of detection and Limit of quantification

Limit of detection (LOD) values for CEFO and OFLO were found to be 0.17 µg/ml and 0.19 µg/ml, respectively and Limit of quantification (LOQ) values for CEFO and OFLO were found to be 0.51 µg/ml and 0.58 µg/ml, respectively (Table 4). These data show that the proposed method is sensitive for the determination of CEFO and OFLO.

Accuracy

The recovery experiment was performed by the standard addition method. The recoveries obtained were 99.87 ± 1.18 % and 99.81 ± 0.27 % for CEFO and OFLO, respectively (Table 2). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 2.

Table 2: Recovery data for the proposed Method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	%Recovery ± S.D. (n=6)
OFLO	1	4	50	99.97 ± 1.28
	2	4	100	99.80 ± 1.50
	3	4	150	99.84 ± 0.77
CEFPO	1	4	50	99.97 ± 0.29
	2	4	100	99.90 ± 0.36
	3	4	150	99.56 ± 0.16

S. D. is Standard deviation and n is number of determinations

Assay of the Pharmaceutical Formulation

The proposed validated method was successfully applied to determine CEFO and OFLO in their tablet dosage form. The result obtained for CEFO and OFLO was comparable with the corresponding labeled amounts (Table 3). The RP-HPLC chromatogram for CEFO and OFLO in sample was recorded and is shown in Figure 6.

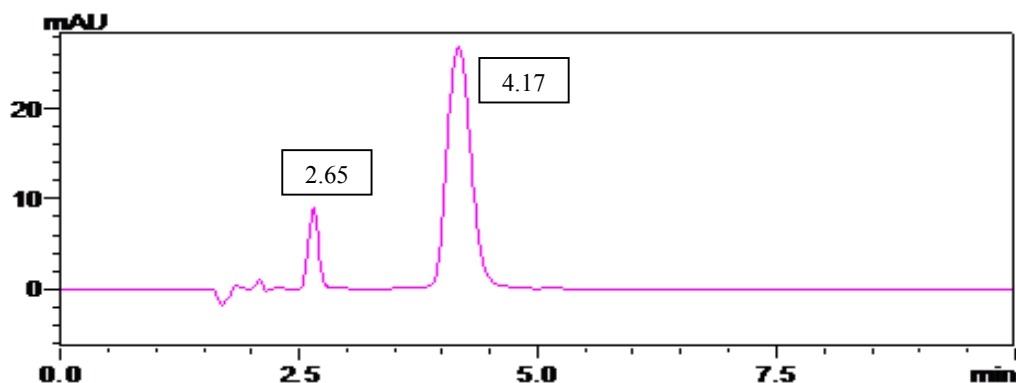


Figure 6: Chromatogram of sample solution of CEFO and OFLO (5 µg/ml) at 236 nm

Table 3: Analysis of formulation of CEFO and OFLO by proposed method (n=6)

Sample No.	Label Claim		Amount Found		% Label Claim	
	CEFO (mg/tab)	OFLO (mg/tab)	CEFO (mg/tab)	OFLO (mg/tab)	CEFO (%)	OFLO (%)
1	200	200	199.74	199.46	99.87	99.73
2	200	200	199.6	200.4	99.80	100.20
3	200	200	199.76	200.36	99.88	100.18
4	200	200	199.56	199.74	99.78	99.87
5	200	200	199.86	199.82	99.93	99.91
6	200	200	199.5	199.58	99.75	99.79
Mean					99.83	99.95
S.D.					0.069	0.199
%RSD					0.069	0.199

RESULTS AND DISCUSSION

A RP-HPLC method was developed and validated for the determination of CEFO and OFLO in tablet dosage forms on a column (C18, 250 X 4.6 i.d., 5µm) with variable wavelength detection at 236 nm. The retention times of CEFO and OFLO was 2.65 min and 4.17 min, respectively.

Linear correlation was obtained between area and concentrations of CEFO and OFLO in the concentration ranges of 1-20 µg/ml and 1-20 µg/ml, respectively. The low RSD values of interday (0.25-1.10 % for CEFO and 0.14-1.33 % for OFLO) and intraday (0.11-0.50 % for CEFO and 0.32-1.24 % for OFLO) at 236 nm, reveal that the proposed method is precise. The limit of detection (LOD) and the limit of quantification (LOQ) for CEFO and OFLO were found to be 0.17 and 0.19 µg/ml and 0.51 and 0.58 µg/ml, respectively. These data show that method is sensitive for the determination of CEFO and OFLO.

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.81 ± 1.18 and 99.87 ± 0.27 for CEFO and OFLO, 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 CEFO and OFLO in their tablet dosage form. The results obtained for CEFO and OFLO 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 CEFO and OFLO in pharmaceutical dosage forms.

Table 4: Regression analysis data and summary of validation parameters for the proposed method

Parameters	RP-HPLC method	
	CEFO	OFLO
Concentration range (µg/ml)	1-20	1-20
Slope	31285	84496
Intercept	1281.4	3811.4
Correlation coefficient	0.9998	0.9996
LOD (µg/ml)	0.17	0.19
LOQ (µg/ml)	0.51	0.58
Accuracy \pm SD (n = 6)	99.81 ± 1.18	99.87 ± 0.27
Repeatability (% RSD, n = 6)	0.28	0.2
Interday (n = 3)	0.25-1.10 %	0.14-1.33 %
Intraday (n = 3)	0.11-0.50 %	0.32-1.24 %
Assay \pm SD (n = 6)	99.83 ± 0.069	99.95 ± 0.199

CONCLUSION

In this proposed method the linearity is observed in the concentration range of 1-20 µg/ml with co-efficient of correlation, (r^2) = 0.9998 and (r^2) = 0.9996 for CEFO and OFLO, respectively at 236 nm.

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the CEFO and OFLO in combined dosage form without any interference of excipients.

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