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Research Article

A SIMPLE AND SENSITIVE RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME AND OFLOXACIN IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, sensitive and validated RP-HPLC method for simultaneous determination of Cefixime and Ofloxacin in combined tablet dosage form has been developed. Separation was carried out on Jasco HPLC system equipped with HiQ-SiL C_8 Neosphere column (150 × 4.6 mm i.d.) and UV/VIS detector using Methanol: 0.025 mM potassium dihydrogen phosphate buffer in ratio of (70:30, v/v) as mobile phase and detection was carried out at 290 nm. Ambient temperature conditions were maintained. Results were linear in the range of 1-10 µg/mL for both the drugs. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

Keywords: RP-HPLC, Cefixime, Ofloxacin, Tablet dosage form.

INTRODUCTION

Cefixime (CEFI) (6*R*, 7R)-7-[2-(2-amino-4-thiazolyl)glyoxyl-amido]-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7-(*Z*)-[*o*-(carboxymethyl)- oxime] trihydrate is third-generation cephalosporin antibiotic[1]. Ofloxacin (OFLOX) chemically 9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1piperazinyl)-7- oxo-7H-pyrido (1, 2, 3-di)-1, 4-benzoxazine carboxylic acid used as an antibacterial[2].

Literature survey reveals High Performance Liquid Chromatographic (HPLC) [3-5], for determination of CEFI in tablet in combination with others drugs. Spectrophotometric method for simultaneous estimation of CEFI with other drugs also reported[6]. HPLC methods have been reported for the determination of OFLOX either in single or in combination with other drugs[7-10]. HPTLC method has been reported for determination of OFLOX in combination with other drugs[11]. Spectrophotometric methods for simultaneous estimation of OFLOX with other drugs also reported[12-14].

No reports were found for the simultaneous estimation of the CEFI and OFLOX in combined tablet dosage form by RP-HPLC method. This paper describes a simple, accurate, sensitive and validated RP-HPLC method for simultaneous quantification of these compounds as the bulk drug and in combined tablet dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines[15].

EXPERIMENTAL

Chemicals and reagents

Working standards of pharmaceutical grade CEFI and OFLOX were obtained as generous gifts from Cipla Pharmaceuticals Ltd. (Solan, H. P., India) used as such without further purification. The pharmaceutical dosage form used in this study was CEFI-O 200 tablets (Accent Pharma, Punducherry, India) labeled to contain 200 mg of CEFI and 200 mg of OFLOX were procured from the local market. Methanol (HPLC grade), Potassium dihydrogen phosphate (AR grade) purchased from Merck specialties Pvt. Ltd. (Mumbai, India) and double distilled water were used in analysis.

Instrumentation and chromatographic conditions

Jasco HPLC system consisting of Jasco PU-2080 plus HPLC pump and UV-2075 plus UV/VIS detector and JASCO Borwin 1.50.8.0 version software was used for analysis. Separation was carried out on Neosphere C_8 (150 x 4.6 mm i.d.) column using Methanol: 0.025 mM Potassium dihydrogen phosphate buffer in ratio of (70:30, v/v) as mobile phase at flow rate of 1.0 mL/min. Samples were injected using Rheodyne injector with 20 µL loop and detection was carried out at 290 nm. All Weighing were done on Shimadzu balance (Model AY-120).

Preparation of standard solutions

Standard stock solutions of pure drugs were prepared separately by dissolving 5 mg of each drug in 10 mL of mobile phase to get concentration of 500 µg/mL. One mL of this stock solution was further diluted to 10 mL with mobile phase to get a working standard solution having concentration 50 µg/mL for both CEFI and OFLOX.

Preparation of sample solution

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 5 mg of OFLOX was transferred to 10 mL volumetric flask containing 7 mL of mobile phase and ultrasonicated for 5 min. The volume was made upto the mark with the mobile phase and filtered through Whatman paper No. 41. 1 mL of filtrate was further diluted to 10 mL of mobile phase to get solution of concentration 50 µg/mL. 0.4 mL of solution was further diluted to 10 mL to get the final concentration 2 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 per tablet was estimated from the respective calibration curves.

System suitability

The system suitability was assessed by six replicate injections of the mixture containing 10 µg/mL of both the drugs. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated as represented in Table 1. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination.

Table 1: System suitability parameters for RP-HPLC method

S. no.	Parameters	CEFI	OFLOX
1	Theoretical plates	2008	2191
2	HETP (cm)	13.38	14.60
3	Resolution*	3.54	4.38
4	Asymmetry factor	1.54	1.42

With respect to previous peak

Method validation

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines[15].

Linearity

Aliquots 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 mL of working standard solutions of CEFI and OFLOX (50 µg/mL each) were transferred in a series of 10 mL volumetric flasks and the volume was made up to the mark with the mobile phase. Five replicates per concentration were injected and chromatograms were recorded. The peak areas were recorded and calibration curve was plotted of peak area against concentration of drug.

Precision

One set of three different concentrations of mixed standard solutions of CEFI and OFLOX were prepared. All the solutions were analyzed thrice, in order to record any intra day variations in the results. For Inter day variations study three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days. The peak areas were recorded and Relative standard deviation (RSD) was calculated for both series of analyses.

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% and 150%. The percentage of recoveries were calculated, results of which are represented in Table 2.

Table 2: Recovery studies of CEFI & OFLOX

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Total amount found (µg/ml)	% Recovery	% RSD
CEFI	2	1	3.07	102.40	0.480
	2	2	4.03	100.91	0.368
	2	3	4.92	98.45	0.238
OFLOX	2	1	3.08	102.95	0.689
	2	2	3.99	99.86	0.129
	2	3	4.94	98.88	0.940

*Avg. of three determinations, R.S.D. is relative standard deviation

LOD and LOQ

LOD and LOQ 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 slope of the calibration plot.

Robustness

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following three factors were selected for change: flow rate of the mobile phase (0.8 ± 0.02 mL/min), a wavelength at which the drugs were recorded (290 ± 2 nm) and mobile phase percentage with respect to methanol (± 2%). One factor at the time was changed to estimate the effect. The solutions containing 6 µg/mL of both the drugs were applied onto the column. A number of replicate analyses (n = 3) were conducted at 3 levels of the factor (-, 0, +). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

For RP-HPLC method different mobile phases were tried and the mobile phase containing Methanol: 0.025 mM Potassium dihydrogen phosphate buffer in ratio of (70:30, v/v) was found to be optimal for obtaining well defined and resolved peaks with mean retention times 3.60 **±** 0.0525 and 6.24 **±** 0.0619 min (Mean ± S.D.) for CEFI and OFLOX respectively. The representative chromatogram of the standard solution of mixture is shown in Fig. 1.

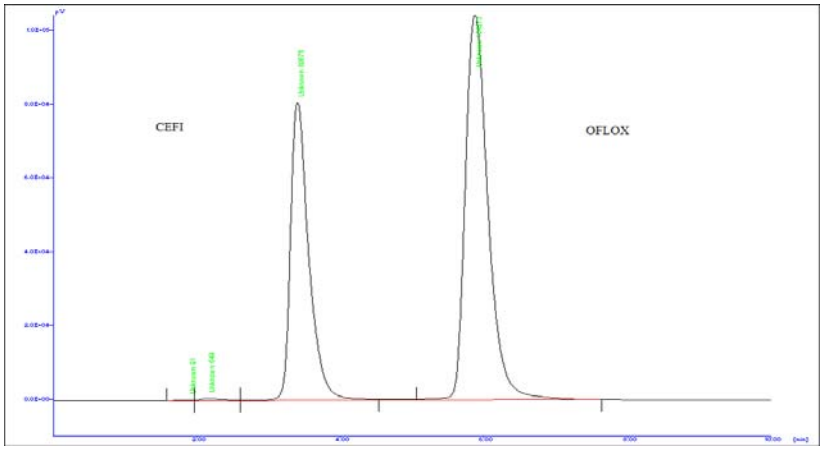

Fig. 1: Representative chromatogram obtained for standard mixture of CEFI (10 µg /mL, 3.6 ± 0.0525 min), OFLOX (10 µg/mL, 6.240 ± 0.0619 min)

Results were found to be linear in the concentration range of 1-10 µg/mL for both CEFI and OFLOX. The correlation coefficients for the plots were 0.999 for CEFI and 0.996 for OFLOX. The proposed method was also evaluated by the assay of commercially available tablets containing CEFI and OFLOX. The % assay was found to be 101.91 ± 0.752 for CEFI and 98.47 ± 1.182 for OFLOX (mean ± S.D., n = 6). The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. Robustness of the method (data not shown), checked after deliberate alterations of the analytical parameters shown no marked changes in the chromatograms (RSD < 2), which demonstrated that the RP-HPLC method developed is robust. The summary of validation parameters of proposed HPLC method is given in Table 3.

Table 3: Summary of validation parameters of proposed RP-HPLC method

Parameters	CEFI	OFLOX
Linearity range (µg/mL)	1-10	1-10
Correlation co-efficient	0.999	0.996
Slope (m)	135452.5	220808.8
Intercept (c)	35109.02	7108.307
LOD[a] (µg/mL)	0.352	0.292
LOQ[b] (µg/mL)	1.069	0.885
Accuracy (% Recovery)	98.45-102.40	98.88-102.95
Precision (% RSD)[c]		
Intra day (n[d] = 3)	0.797	0.867
Inter day (n = 3)	1.26	1.33

[a]LOD = Limit of detection; [b]LOQ =Limit of quantitation; [c]RSD = Relative standard deviation; [d]n = Number of determination

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of CEFI and OFLOX in combined tablet dosage form.

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