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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Cefixime Trihydrate and Ofloxacin in Tablets

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Abstract: This research manuscript describe simple, sensitive, accurate, precise and repeatable RP-HPLC method for the simultaneous determination of Cefixime Trihydrate and Ofloxacin in tablet dosage form. The sample was analyzed by reverse phase C18 column (Phenomenex C18, 5µ, 250 mm × 4.6 mm) as stationary phase; methanol : water : phosphate buffer, pH 4.9 (60: 20 : 20 , v/v/v) as a mobile phase at a flow rate of 1.0 ml/min. Quantification was achieved with PDA detector at 290 nm. The retention time for Cefixime Trihydrate and Ofloxacin was found to be 3.30 and 5.00 min, respectively. The linearity for both the drugs was obtain in the concentration range of 2.0-20 µg/ml with mean accuracies 99.48 ± 0.83 and 99.52 ± 0.91 for Cefixime Trihydrate and Ofloxacin, respectively. The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from tablet excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.
Key words: Cefixime trihydrate, Ofloxacin, RP-HPLC, Combined dosage forms, Method validation.

INTRODUCTION

Cefixime trihydrate (CEFI), [6R, 7R] – 7- [[(2Z)- 2-(2-amino thiazole- 4-yl)- [(carboxy methoxy) imino] aetyl] amino]-3-ethenyl -8-oxo 5-thia 1-aza bicyclo [4.2.0] oct-2- ene-2 carboxylic trihydrate is a third generation orally acting cephalosporin antibiotic[1]. Ofloxacin (OFLO), 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 is a floroquinolone antibiotic[2]. This combination is used in the treatment of typhoid fever, urinary tract infection, respiratory tract infection, nosocomial infections, soft tissue infections, surgical prophylaxis and intra-abdominal infections[3]. Literature survey reveals spectrophotometric[4], HPLC[5] and HPTLC[6] methods for determination of CEFI in pharmaceutical dosage forms as well as in biological fluids. Literature survey reveals spectofluorimetric[7-8] and HPLC[9-10] methods for determination of OFLO in pharmaceutical dosage forms as well as in biological fluids. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of CEFI and OFLO in their combined dosage forms. Literature survey does not reveal any simple RP-HPLC or other method for simultaneous estimation of CEFI and OFLO in combined dosage forms. The present communication describes simple, sensitive, accurate and precise RP-HPLC method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS & METHODS

Apparatus

The chromatography was performed on a Shimadzu (Columbia, MD) RP-HPLC instrument (LC-2010CHT) equipped with PDA detector and LC-solution software, Phenomenex (Torrance, CA) C18 column (250 mm × 4.6 mm id, 5µm particle size) was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used in the study.

Reagents and materials

CEFI 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, triple distilled water (S. D. Fine Chemicals Ltd., Mumbai, India) used were of HPLC grade. Potassium dihydrogen phosphate, sodium hydroxide, sulphuric acid (S.D Fine Chemicals Ltd., Mumbai, India) used were of AR grade. Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India) and Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

Preparation of buffer solution

Potassium dihydrogen phosphate buffer pH = 4.9. Accurately weighed 40 g of potassium dihydrogen phosphate and 1.2 gm of sodium hydroxide was transferred to a 1000 ml volumetric flask, dissolved in and diluted up to mark with HPLC grade water. pH = 4.9 was adjusted with 1 M H_2SO_4.

Preparation of standard stock solutions

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

Preparation of calibration curve

Aliquots (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 1.0 ml) of mixed standard solutions (equivalent to 2, 4, 6, 8, 10, 14, 16 and 20 µg/ml of CEFI and OHLO, each) were transferred in a series of 5 ml volumetric flasks, and the volume was made up to the mark with methanol. An aliquot (20 µl) of each solution was injected under the operating chromatographic condition as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentration, and the regression equations were calculated. Each response was average of three determinations.

Preparation of sample solution

For determination of the content of CEFI and OFLO in tablets; twenty tablets were weighed and the average weight was calculated. The tablet powder equivalent to 10 mg of CEFI and 10mg of OFLO were weighed and transferred to 100 ml volumetric flask. Methanol (50 ml) was added and sonicated for 20 min. The flask was allowed to stand for 5 min at room temperature and the volume was adjusted up to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41. The solution was suitably diluted with mobile phase to get a final concentration of 10 µg/ml of CEFI and 10 µg/ml of OFLO. An aliquot (20 µl) of sample solution was injected under the operating chromatographic condition as described above and responses were recorded. The analysis procedure was repeated three times with tablet formulation.

Method Validation

The method was validated in compliance with ICH guidelines.

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of CEFI and OFLO by the standard addition method. Known amounts of standard solutions of CEFI and OFLO were at added at 50, 100 and 150 % level to prequantified sample solutions of CEFI and OGLO (5 µg/ml for both drug). The amounts of CEFI and OFLO were estimated by applying obtained values to the respective regression line equations.

Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of CEFI and OFLO (10 µg/ml for both drugs) without changing the parameters.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of CEFI and OFLO (4, 8 and 12 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

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[11]
LOD = 3.3 × σ/S
LOQ = 10 × σ/S

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Robustness
The robustness was studied by analyzing the same samples of CEFI and OFLO by deliberate variations in the method parameters. The change in the responses of CEFI and OFLO were noted. Robustness of the method was studied by changing the extraction time of CEFI and OFLO from tablet dosage forms by ± 2 min, composition of mobile phase by ± 2 % of organic solvent, flow rate by ± 2 ml/min and column oven temperature by± 2 °C. The parameters used in system suitability test were asymmetry of the chromatographic peak, peak resolution and theoretical plates, as RSD of peak area for replicate injections.

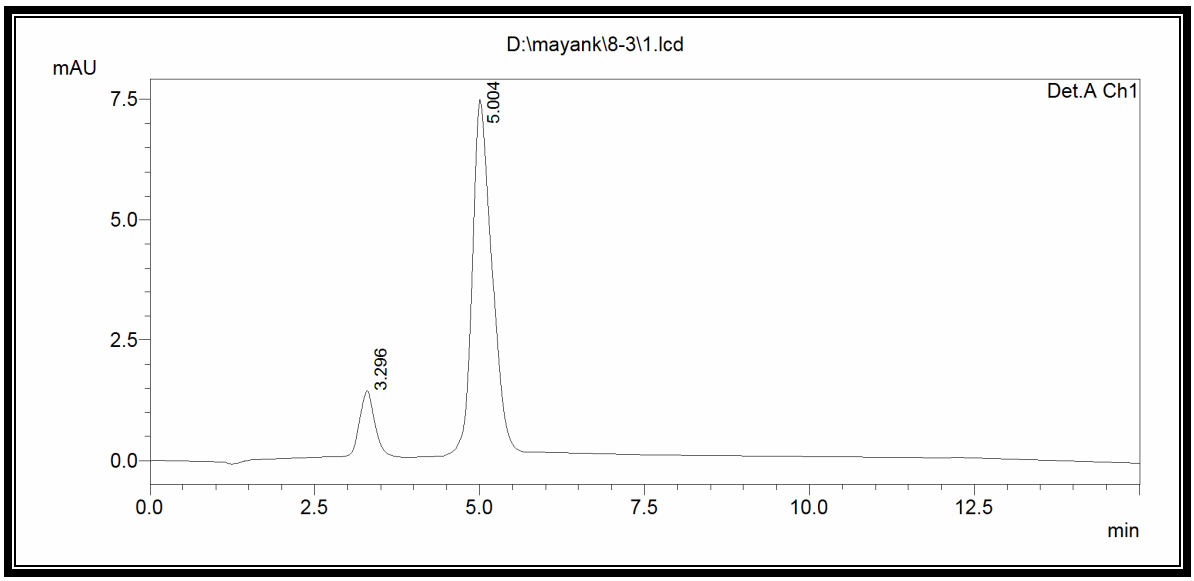

Figure 1. Chromatogram of CEFI (10 µg/ml) and OFLO (10 µg/ml) at 290 nm

Table 1 Regression analysis data and summary of validation parameter for the proposed RP-HPLC method

Parameters	RP-HPLC method	
	CEFI	OFLO
Concentration range (µg/ml)	2-20	2-20
Slope	38044	11560
Intercept	64533	58797
Correlation coefficient	0.9980	0.9998
LOD[a] (µg/ml)	0.52	0.46
LOQ[b] (µg/ml)	1.68	1.53
Accuracy (n[c] = 6)	99.48 ± 0.83	99.52 ± 0.91
Repeatability (% RSD[d], n = 6)	0.28	0.2
Precision (%RSD)		
Interday (*n* = 3)	0.25-1.10 %	0.14 – 1.33 %
Intraday (*n* = 3)	0.11-0.50 %	0.32 – 1.24 %

a = Limit of detection b = Limit of quantification c = number of determinations
d = Relative standard deviation

Table 2 System suitability test parameters for CEFI and OFLO for the proposed RP-HPLC method

Parameters	CEFI ± RSD (n = 6)	OFLO ± RSD (n = 6)
Retention time (min)	3.30 ± 1.25	5.00 ± 0.33
Tailing factor	1.14 ± 1.09	1.49 ± 0.42
Asymmetry factor	1.23 ± 0.89	1.31 ± 0.65
Theoretical plates	2597 ± 1.79	3860 ± 1.24
Resolution	4.55 ± 0.76	

Table 3 Recovery data for the proposed method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± S.D. (n=6)
CEFI	I	5	50 %	99.20 ± 0.75
	II	5	100 %	100.20 ± 1.15
	III	5	150 %	99.04 ± 0.59
OFLO	I	5	50 %	98.40 ± 0.64
	II	5	100 %	100.8 ± 0.53
	III	5	150 %	99.36 ± 1.56

Table 4 Analysis of marketed formulation of CEFI and OFLO by proposed RP-HPLC method (n = 3)

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n=3)	
	CEFI	OFLO	CEFI	OFLO)	CEFI	OFLO
I	200	200	198.60	200.56	99.30±1.03	100.28±1.12
II	200	200	199.52	198.87	99.76±0.74	99.43±1.25

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for CEFI and OFLO was obtained with a mobile phase comprising of methanol: water: phosphate buffer, pH 4.9 (60: 20: 20, v/v/v) at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 290 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Figure 1). The retention time for CEFI and OFLO were found to be 3.30 and 5.00 min, respectively (Figure 1). Linear correlation was obtained between peak area versus concentrations of CEFI and OFLO in the concentration ranges of 2-20 µg/ml (Table 1). The method was found to be specific as no significant changes in the responses of CEFI and OFLO was observed after 24 h. The mean recoveries obtained were 99.48 ± 0.83 % and 99.52 ± 0.91 % for CEFI and OFLO, respectively (Table 1 and 3), which indicates accuracy of the proposed method. The % RSD values for CEFI and OFLO were found to be <2 %, which indicates that the proposed method is repeatable. 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 %) variations for CEFI and OFLO, respectively, reveal that the proposed method is precise. LOD values for CEFI and OFLO were found to be 0.52 µg/ml and 0.46 µg/ml, respectively and

LOQ values for CEFI and OFLO were found to be 1.68 µg/ml and 1.53 µg/ml, respectively (Table 1). These data show that the proposed method is sensitive for the determination of CEFI and OFLO. The results of system suitability testing are given in Table 2. The amount of CEFI and OFLO present in the sample solutions were determined by fitting the responses into the regression equations of the calibration curve for CEFI and OFLO, respectively and the results obtained were comparable with the corresponding labeled claim (Table 4).

CONCLUSION

In this proposed method the linearity is observed in the concentration range of 2-20 µg/ml with co-efficient of correlation, (r^2) = 0.9980 and (r^2) = 0.9998 for CEFI and OFLO, respectively at 290 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 CEFI and OFLO in combined dosage form without any interference of excipients.

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