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RPHPLC-DAD METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND OFLOXACIN IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, sensitive and validated RP-HPLC method involved with Diode Array Detection was developed for simultaneous determination of Cefiximetrihydrateand Ofloxacin in combined tablet dosage form has been developed. The sample was analyzed by reverse phase Teknokroma (Kromosil) C8 (150 x 0.6 mm i.d.) column as stationary phase with PDA detection at 285 nm. The separation was carried out using a mobile phase consisting of 0.01 %v/v Triethylaminephosphate buffer of pH 5.2 and Acetonitrile in the ratio of 80: 20 at a flow rate of 0.8 ml/min. at ambient temperature conditions. The method was validated for Accuracy, Precision, Linearity, Ruggedness, and Robustness. The method was successfully applied on tablet dosage form.

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

INTRODUCTION

Cefixime trihydrate(CEFI) (6*R*,7R)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-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 ofloxacin.[1] (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. 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.

Literature survey reveals High Performance Liquid Chromatographic (HPLC). [2.4] for determination of CEFI in tablet in combination with others drugs.[5,6] Spectrophotometric method for simultaneous estimation of CEFI with other drugs also reported[7]. HPLC methods have been reported for the determination of OFLOX either in single or in combination with other drugs [1-6]. HPTLC method has been reported for determination of OFLOX in combination with other drugs. [8] Spectrophotometric methods for simultaneous estimation of OFLOX with other drugs also reported.[9] 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 USP guideline Stucture are shown in figure 1and 2. [10]

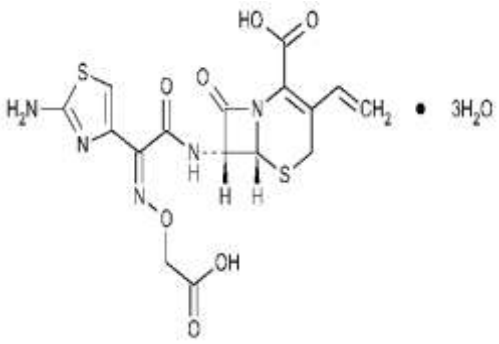

Figure 1: Structure of CefiximeTrihydrate

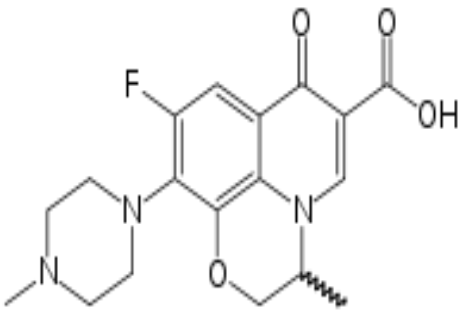

Figure 2: Structure of Ofloxacin

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 ZIFI-O 100 tablets (Akum Pharmaceutical, Haridwar India) labeled to contain 100 mg of CEFIXIME TRIHYDRATE and 100 mg of OFLOXACIN were procured from the local market. Acetonitrile (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

Water HPLC system consisting of Auto sampler& 600 controllers with windows based Empower software996 and PDA detector was used for analysis. Separation was carried out

onTeknokroma (Kromosil) C8 (150 x 0.6 mm i.d.) column using 0.01mMPotassium dihydrogen phosphate buffer : Acetonitrile in ratio of (80:20, v/v) as mobile phase at flow rate of 0.8 mL/min. and sample volume 20µl.Detection was carried out at 285 nm. All Weighing were done on Citizen Balance (Model cY-104).

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 up to 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.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 three times and the amount of each drug present per tablet was estimated from the respective calibration curves. Typical Chromatogram of Cefixime (RT =2.867 min) and Ofloxacin (RT= 4.551 min) are shown in figure 3.

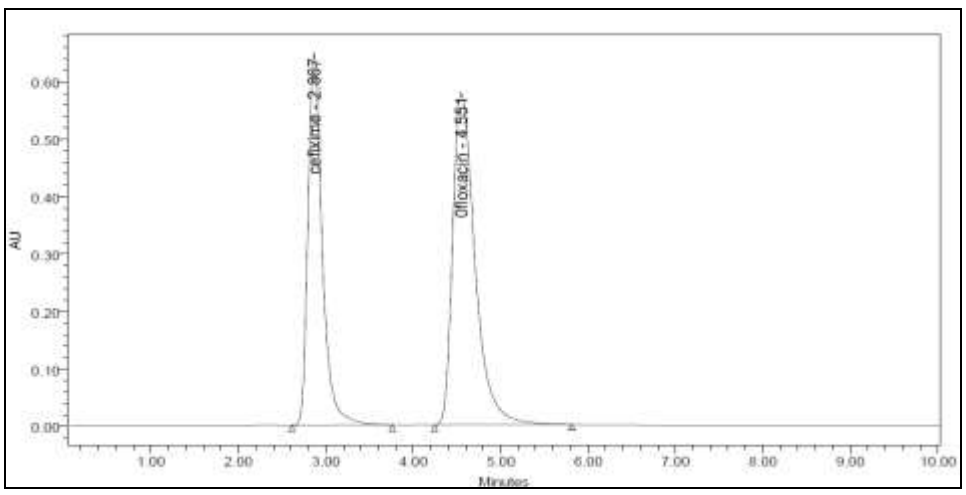

Figure 3: Typical Chromatogram of Cefixime (RT =2.867 min) and Ofloxacin (RT= 4.551 min).

System suitability

The system suitability was assessed by three 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 shown in Table 1. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination.

Table 1: System Suitability Data

Parameters	Cefixime trihydrate	Ofloxacin
Theoretical plates	2605.78	2720.24
Symmetry Factor	1.411	1.575
Tailing factor	1.41	1.58

Method validation

The method was validated for linearity, accuracy, precision and robustness, in accordance with USP guidelines. [2]

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 80%, 100% and 120%. The percentage of recoveries were calculated, results of which are represented in Table 2.

Table 2: Accuracy study data

Drug	Amt. taken (µg/ ml)	Amt. added%	% Recovery	% RSD
CEFI	5	80	100.27	0.17
	5	100	99.89	0.056
	5	120	100.56	0.65
OFLO	5	80	100.35	0.41
	5	100	100.06	0.29
	5	120	99.65	0.35

Linearity

Different conc. of CEFI (20-100µg/ml) and OFLO (20-100 µg/ml) were prepared from stock solution of respective API. 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. Specimen chromatogram and result of linearity shown in figure 4 and table 3 respectively. linearity plots are shown in figure 5and 6.

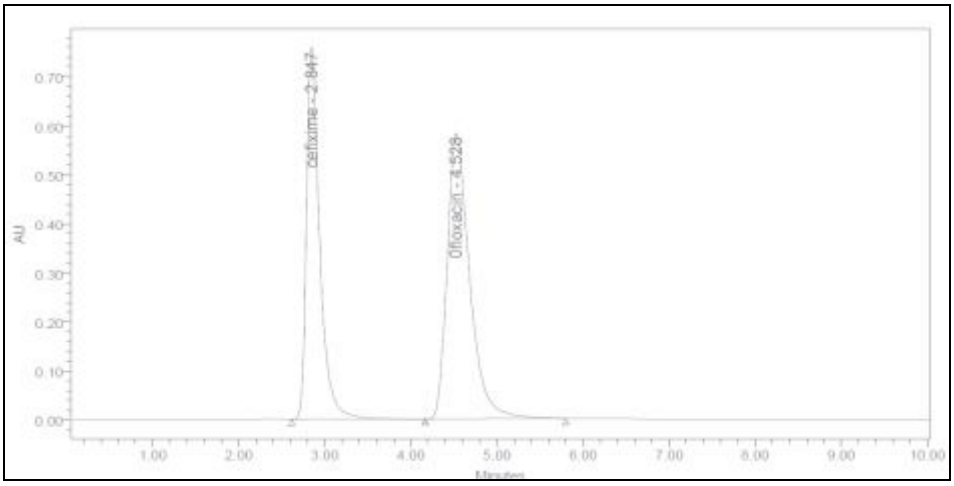

Figure 4: Specimen Chromatogram of linearity

Table 3: Result of linearity studies.

Level	CEFI		OFLO	
	Concentration (µg/mL)	Average Area response	Concentration (µg/mL)	Average Area response
Linearity 20%	20	429713.5	20	1042574.5
Linearity 40%	40	847915	40	2042031
Linearity 60%	60	1370996	60	3294563
Linearity 80%	80	1806280.5	80	4367913
Linearity 100%	100	2068258.5	100	5004022
Correlation Coefficient	0.993		0.994	
Slope	21420		51784	
Intercept	16189		35994	

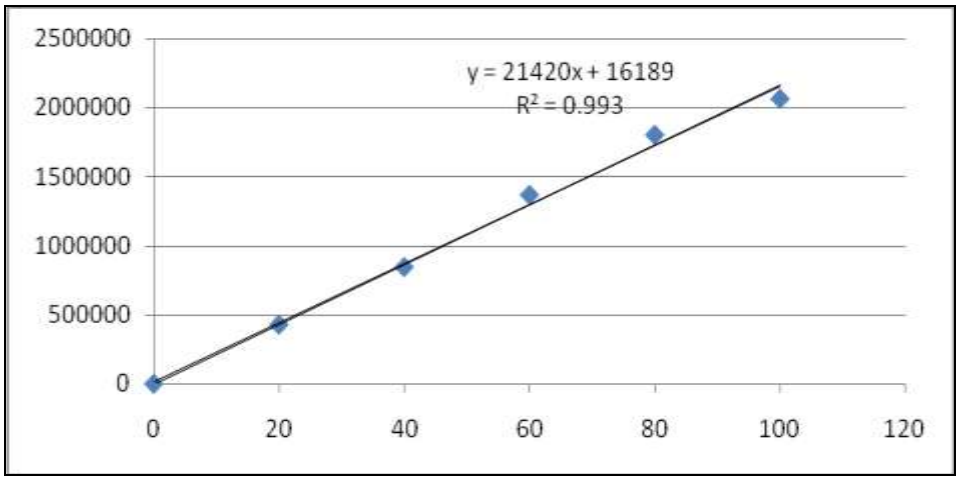

Figure 5 : Linearity plot for CEFI

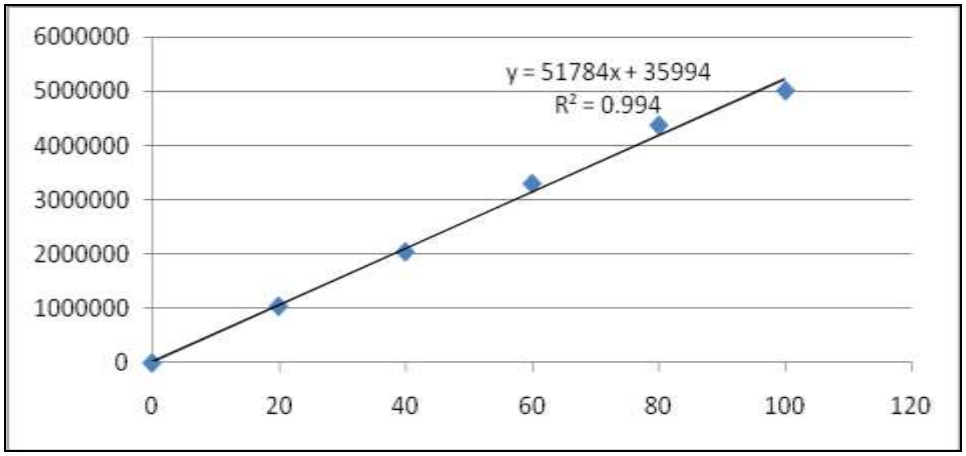

Figure 6 : Linearity plot for OFLO.

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 intraday 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. Chromatograms of precision are shown in figure 7.

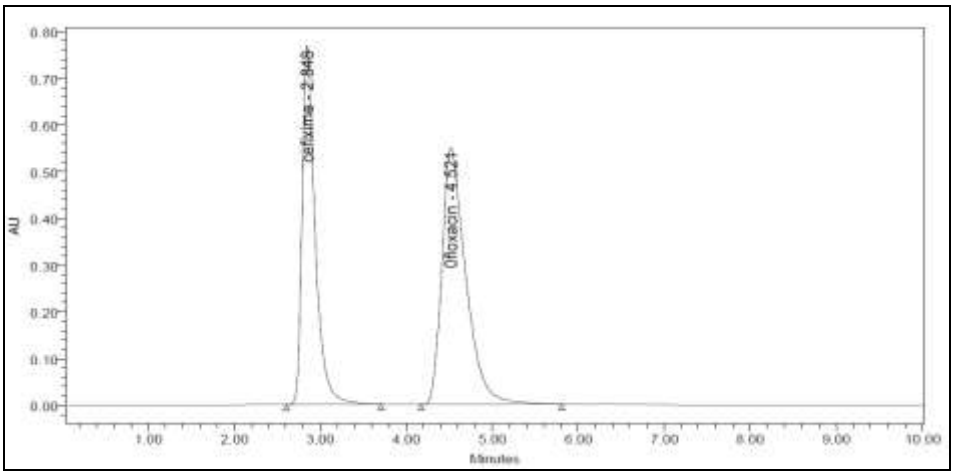

Figure 7: Chromatogram of precision.

Ruggedness (Intermediate precision)

Inject Standard preparation and Sample preparations into the chromatograph, record the chromatograms and measure the peak responses for the major peaks. Check the system suitability and record the results in the table 4, 5 and figure 8.

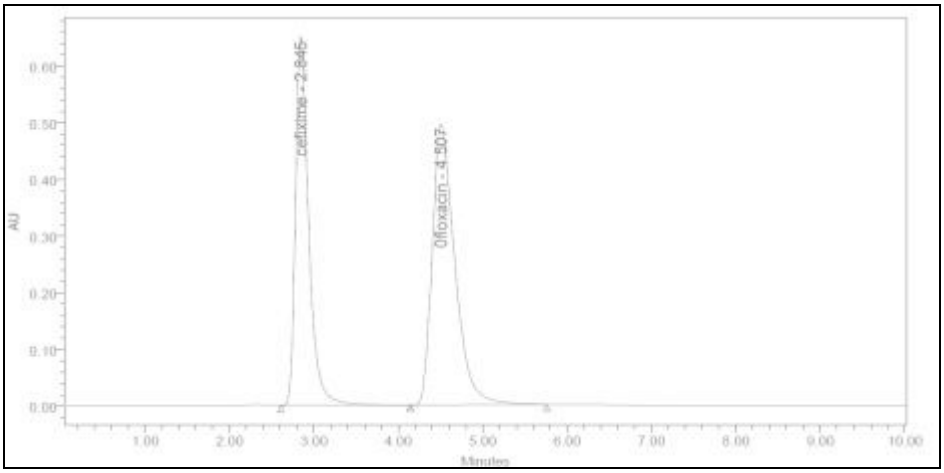

Figure 8: Chromatogram of intermediate precision

Table 4: Intermediate Precision Studies.

Injection No.	Area Response	
	CEFI	OFLO
1	1367912	3291892
2	1374080	3297234
3	1372591	3294132
Average	1371528	3294419
SD	2627.935	2190.306
% RSD	0.191606	0.066485

Table 5: Precision data.

Sr. No	% Assay		% Assay	
	Set-I	Set-II	Set-I	Set-II
	CEFI		OFLO	
1	100.1361	100.1361	100.0892	100.0892
2	99.93346	99.93346	99.87798	99.87798
3	99.99599	99.99599	99.97831	99.97831
Average	100.240		99.97882	
Over all SD	0.264381		0.07705	
Over all % RSD	0.263747		0.077074	

SET – I: Method Precision data **SET – II: Intermediate precision data**

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 ± 0.1 mL/min), a wavelength at which

the drugs were recorded (285 ± 5 nm) It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. Robustness chromatogram showed in figure 9&10. System suitability data for Robustness showed in table 6**.**

1) Flow variation: 0.7ml

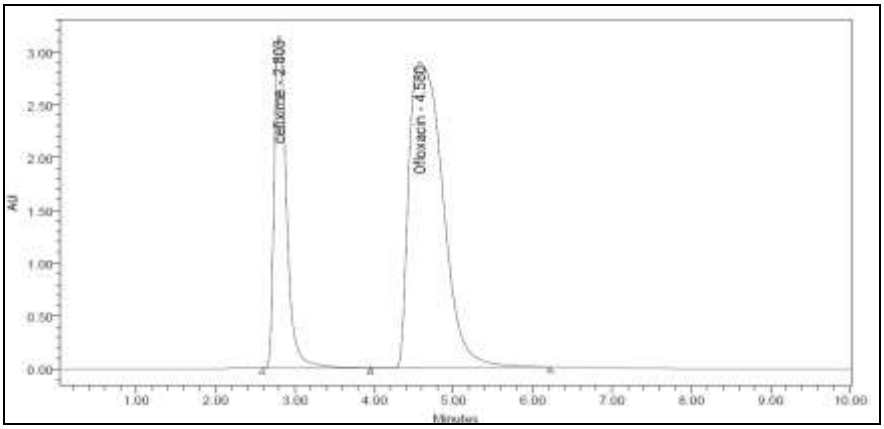

Figure 9: Chromatograms of change in 0.7ml flow rate.

2) Flow variation:0.9 ml

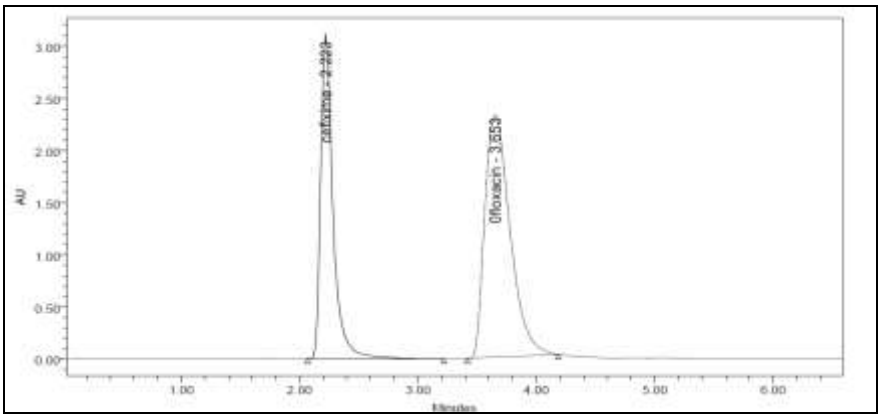

Figure 10: Chromatograms of change in 0.9 ml flow rate.

Table 6: System suitability data for robustness.

Sn.	System Suitability parameter		As Such	Flow		Wavelength.		limits
				-10 %	+10%	- 5 nm	+ 5 nm	
1	The % RSD of peak area response for three replicate injections	CEFI	0.1	1.96	0.02	0.59	0.33	NMT 2.0
		OFLO	0.3	0.97	0.1	0.53	0.50	
2	Theoretical plates	CEFI	2605	2141	2348	2445	2447	NLT 2000
		OFLO	2720	1761	2068	2283	2278	
3	Tailing factor	CEFI	1.40	1.54	1.59	1.41	1.45	NMT 2.0
		OFLO	1.81	1.77	1.5	1.51	1.50	

RESULT AND DISCUSSION

For RP-HPLC method different mobile phases were tried and the mobile phase containing Acetonitrile: 0.01 mM Potassium dihydrogen phosphate buffer in ratio of (20:80, v/v) was found to be optimal for obtaining well defined and resolved peaks with mean retention times 2.867 and 4.551 min (Mean ± S.D.) for CEFI and OFLOX respectively.

Results were found to be linear in the concentration range of 20-100 µg/mL for both CEFI and OFLOX. The correlation coefficients for the plots were 0.993 for CEFI and 0.994 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 100.24 for CEFI and 99.97 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 7.

Table 7: Summary of validation parameters of proposed RPHPLC method.

Parameters	CEFI	OFLOX
Linearity range (µg/mL)	20-100	20-100
Correlation co-efficient	0.993	0.994
Slope (m)	21420	51784
Intercept (c)	16189	35994
Accuracy (% Recovery)	100.52	100.02
Precision (% RSD)	0.263747	0.077074

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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