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RESEARCH ARTICLE

Simultaneous Estimation of Cefixime and Dicloxacillin in Bulk and Tablet Formulation by RP-HPLC Method

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Summary

Present study reports development and validation of simple, economic, selective, precise, and accurate RP high-performance liquid chromatography (RP-HPLC) method for the analysis of cefixime and dicloxacillin in bulk drug and pharmaceutical formulations. The developed method has shown good resolution for cefixime, dicloxacillin and formulation excipients present in tablets. A mixture of 5 mM Phosphate Buffer (pH 5.4 adjusted with ortho-phosphoric acid): acetonitrile: methanol (42: 55: 3 v/v/v) was used to elute the drug component. HPLC analysis of cefixime and dicloxacillin was carried out at a wavelength of 225 nm with flow rate of 1.0 mL min^{-1}. The method was quantitatively evaluated in terms of linearity, precision, accuracy (recovery), selectivity and robustness as per standard guidelines. The method is simple, convenient and suitable for the analysis of cefixime and dicloxacillin in bulk and pharmaceutical formulations.

Key Words: *Reverse-phase HPLC, Cefixime, Dicloxacillin, ICH guidelines.*

Sefiksim ve Dikloksasilin'in Ham Madde ve Farmasötik Formülasyonlardan TF-HPLC Yöntemi ile Eşzamanlı Tayini

Özet

Bu çalışmada sefiksim ve dikloksasilin'in farmasötik formülasyonlar ve ham madde olarak ters faz yüksek performanslı sıvı kromatografisi yöntemi ile basit, ekonomik, seçici, tam ve doğru analiz edilmesine yönelik analitik yöntem geliştirilmesi ve validasyonu raporlanmıştır. Geliştirilen bu yöntemde, sefiksim, dikloksasilin ve tabletler içerisinde yer alan diğer eksipiyanlar için iyi resolüsyon göstermektedir. Etkin maddeleri elüe etmek için 5 mM fosfat tamponu (orto-fosforik asit ile pH 5.4'e ayarlanmış):asetonitril: metanol (42: 55: 3; h/h/h) karışımı kullanılmıştır. Sefiksim ve dikloksasilin'in HPLC analizi 225 nm dalga boyunda 1.0 mL dk^{-1} akış hızında gerçekleştirilmiştir. Standart rehberler doğrultusunda yöntem doğrusallık, kesinlik, doğruluk (geri kazanım), seçicilik ve sağlamlık bakımından kantitatif olarak değerlendirilmiştir. Yöntem, sefiksim ve dikloksasilin'in ham madde ve farmasötik formülasyonlardan tayini açısından basit, elverişli ve uygulanabilirdir. .

Anahtar Kelimeler: *Ters-faz HPLC, Sefiksim, Dikloksasilin, ICH rehberleri*

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INTRODUCTION

Cefixime, (6R,7R)-7-[2-(2-amino-4-thiazolyl) glyoxylamido] -8-oxo3-vinyl-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (CFX) is a third generation cephalosporin antibiotic. CFX has broad and potent activities against various pathogens especially gram negative organisms. CFX is given orally in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory tract infection and urinary tract infection (1,2). Dicloxacillin, (2S,5R,6R)-6-{[3-(2,6-dichlorophenyl) -5-methyl-oxazole-4-carbonyl] amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid (DLX) is used to treat infections caused by susceptible gram-positive bacteria (14). The combination therapy is of choice in treating various bacterial infections.

Several analytical methods have been reported for analysis of CFX and DLX in biological fluid as single drug candidate, however very few methods have been reported for simultaneous estimation of CFX and DLX in combination with other antibiotics from biological samples. Literature survey revealed reports on analytical methods such as UV-VIS, HPLC, LC-MS, LC-MS-MS and HPTLC for the determination of CFX (3-15) and DLX (14-28) alone or in combination with other antibiotic (formulations and in biological samples). Internal standard (IS) is generally used to check the interference of formulation excipients into the developed method which ultimately governs the specificity of the method of analysis. Dhoka et. al. (14) has reported an HPLC method for simultaneous estimation of CFX and DLX using methanol: 0.01 M phosphate buffer (75: 25, v/v), pH 3 as eluting solvent. The aqueous method reported by Dhoka et. al. (14) and Kathiresan K et. al. (15) was without the use of an internal standard, which was found to be the limitation of method. The present investigation was carried out in the view of establishing a simple, precise, economic and accurate isocratic reverse phase HPLC method for simultaneous estimation of CFX and DLX in bulk and tablet formulation using an internal standard.

MATERIALS AND METHODS

Instrumentation

Isocratic high pressure liquid chromatography Cyberlab-chrom-HPLC, V 4.0 (Cyberlabs, USA) with an LC-P-100 pump, variable wavelength programmable UV/Vis detector LC-UV 100 and operating software Cyberstore version 4-0512-039 were used. Chromatographic separation was carried out by reverse phase capcell pak C_{18} DDS5 column (4.6 mm x 250 mm particle size 5 µm).

Chromatographic Condition

The mobile phase consisting of 5 mM phosphate buffer (pH 5.4): acetonitrile:methanol (42:55:3), (v/v/v), was degassed and filtered by using Millipore vacuum filter system equipped with 0.45 µm membrane filter. Chromatography was performed at an ambient temperature by pumping the mobile phase with a flow rate of 1.0 mL min^{-1}. The column effluence was monitored at 225 nm.

Chemicals and Reagents

Cefixime, dicloxacillin (Blok Pharma Pvt. Ltd, Kolhapur), Ezetimibe (Smruthi Organics, Solapur), Acetonitrile (Merck Chemicals) and all other chemicals used were of analytical grade. Double distilled water was used for preparing mobile phase solutions. The tablet of cefixime + dicloxacillin (commercial name–HIFEN-LXX 200) was obtained from a local market in Gulbarga, Karnataka (India).

Preparation of the standard solution

Stock solution containing 1mg mL^{-1} each of CFX, DLX and ezetimibe (IS) was prepared in mobile phase. IS was further diluted with mobile phase to get 10 µg mL^{-1}final concentration. Further the stock solutions of CFX and DLX were diluted with mobile phase to obtain various concentrations of 1, 5, 10, 15, 20 and 25 µg mL^{-1}. Phosphate buffer (5 mM) was prepared in water and adjusted to pH 5.4 with ortho-phosporic acid. Stock solutions were stored in a freezer set to –20°C.

Preparation of Tablet Formulations

Powder of twenty tablets (HIFEN-LXX 200), each containing 200 mg CEF and 500 mg DLX, were weighed. A quantity of powder equivalent to 20 mg of CFX and 50 mg of DLX were taken in different 10 mL volumetric flasks containing about 5 mL mobile phase for analysis and sonicated for 15 min. After sonication the volume was made up to the mark with the same solution to obtain sample stock solution of CFX (2000

µg mL^{-1}) and DLX (5000 µg mL^{-1}). Further, solution was filtered using 0.45 µm membrane filter. The filtrate (0.05 mL) was quantitatively transferred to a 10 mL volumetric flask, 1 mL internal standard solution having concentration of 10 µg mL^{-1} was added, and the solution was diluted to volume with mobile phase.

Method Validation

The chromatographic conditions applied in the present manuscript were found to be appropriate for the quantitative determination. After optimization of the analytical conditions, certain parameters such as linearity, precision, accuracy (recovery), selectivity, and robustness were evaluated for the method validation (29, 30).

System Suitability Test

System-suitability tests were performed according to USP 24/NF 19 to confirm the reproducibility of the equipment adequate for the analysis (30). The test was performed before analysis of each batch of sample to ensure the reproducibility of the chromatographic system. The criteria selected are based on the actual performance of the method, as determined during its validation. These parameters include relative standard deviation (%RSD) of retention times, tailing factor, theoretical plate and asymmetry for repetitive injections.

Linearity

The linearity was studied using six concentrations at 1, 5, 10, 15, 20 and 25 µg mL^{-1} of CFX and DLX. Linearity experiment was performed three times to check the detector's response to be linear in function with various concentrations of drugs (1 to 25 µg mL^{-1}). The working standards were prepared by adding different concentrations of CFX, DLX and fixed concentrations of IS (10 µg mL^{-1}) solution to obtain the required concentration range and then injecting into the HPLC system. The calibration curves were constructed by plotting peak area versus concentrations of CFX and DLX, and the regression equations were calculated.

Accuracy (% Recovery)

Precision and accuracy can often be enhanced by using an appropriate IS for HPLC method, which also serves to correct fluctuations in the detector response. The accuracy study was performed using the standard addition method. The pre-quantified sample solution of CFX (2.00 µg mL^{-1}) and DLX (5.00 µg mL^{-1}) were spiked with an extra 0, 50, 100, and 150% of the standard CFX and DLX respectively. These mixtures were analyzed by the developed method. The experiment was performed in triplicate. The percentage recovery of the samples, %RSD and the percentage were calculated at each concentration level.

Precision

Intra-day and inter-days precision values were estimated at three different concentrations (1, 15 and 25 µg mL^{-1}) of CFX and DLX three times on the same day and on three separate days to obtain the relative standard deviation (%RSD).

LOD and LOQ

Several approaches are given in ICH guidelines to determine the detection (LOD) and quantification (LOQ) limits. In this study, LOD and LOQ were based on the standard deviation of the response and slopes using signal-to-noise ratio as per ICH guidelines (29).

Robustness

The robustness of the developed method was performed to evaluate the influence of a small but deliberate variation in the chromatographic conditions. The robustness of the method was determined by changing the flow rate (0.9 and 1.1 mL min^{-1}) of the mobile phase, pH (4.5 and 4.7) of phosphate buffer and percentage of the buffer in mobile phase.

Selectivity

The selectivity was verified by checking comparison of chromatogram obtained from standard, sample and the corresponding placebo.

RESULTS AND DISCUSSIONS

For the RP-HPLC method, chromatographic conditions were optimized to achieve a good resolution and a peak shape for CFX and DLX. The mobile phase was optimized to provide sufficient selectivity towards the drugs. Phosphate buffer

contributed high sensitivity and selectivity when compared with other buffers. Methanol and acetonitrile as organic components resulted in better sensitivity but variation in the amount of methanol and acetonitrile in the mobile phase affected resolution and runtime. Variation of mobile phase pH resulted in bad peak shape, so the pH of buffer was adjusted to 5.4 with ortho-phosphoric acid. Retention time of approximately 2.83 ±0.08, 3.97 ±0.09 and 6.65 ±0.07 min were consistently observed for CFX, DLX and IS respectively, throughout all analytical runs.

A typical chromatogram for 1 µg mL^{-1} of CFX, DLX and IS from standards are shown in Figure 1. Figure 2 illustrates chromatogram of CFX (5 µg mL^{-1}), DLX (25 µg mL^{-1}) and ezetimibe as IS from tablet.

System Suitability Test

The USP suggests that system suitability tests could be performed prior to analysis (30). Typically, at least two of these criteria are required to demonstrate system suitability for the proposed method. Some of the tests were carried out on fresh standard solutions prepared including drug compounds. Tailing factors were 0.5429 ±0.0225 for CFX and 0.7362 ±0.0247 for DLX. The theoretical plate number (*N*) was 5274.83 ±164.0580 for CFX and 4042.00 ±97.6893 for DLX respectively. The chromatographic conditions described ensured adequate retention and asymmetry for drug compounds. The retention time of drug CFX and CLX were 2.8350 ±0.0083 and 3.9433 ±0.0081 min, respectively. Asymmetry was found to be 0.7131 ±0.0370 for CFX and 0.9306 ±0.0525 for DLX. The variation in retention time for six replicate injections of drug compounds gave %RSD of 0.2951% for CFX and 0.2069% for DLX. The results obtained from the system suitability tests (Table 1) satisfy the USP and ICH standards (29, 30).

Specificity

There is no interference from impurity, excipients or additives were found as additives in tablets are practically insoluble in mobile phase, whereas the active constituents are soluble.

Linearity

CFX and DLX demonstrated that the calibration curves were linear in the concentration range from 1-25 µg mL–1. Linearity of the calibration curves were validated by the value of correlation coefficients of the regression (r2). The correlation coefficients were 0.9998 for CFX and 0.9999 for DLX. The results of the linearity experiment are listed in Table 2.

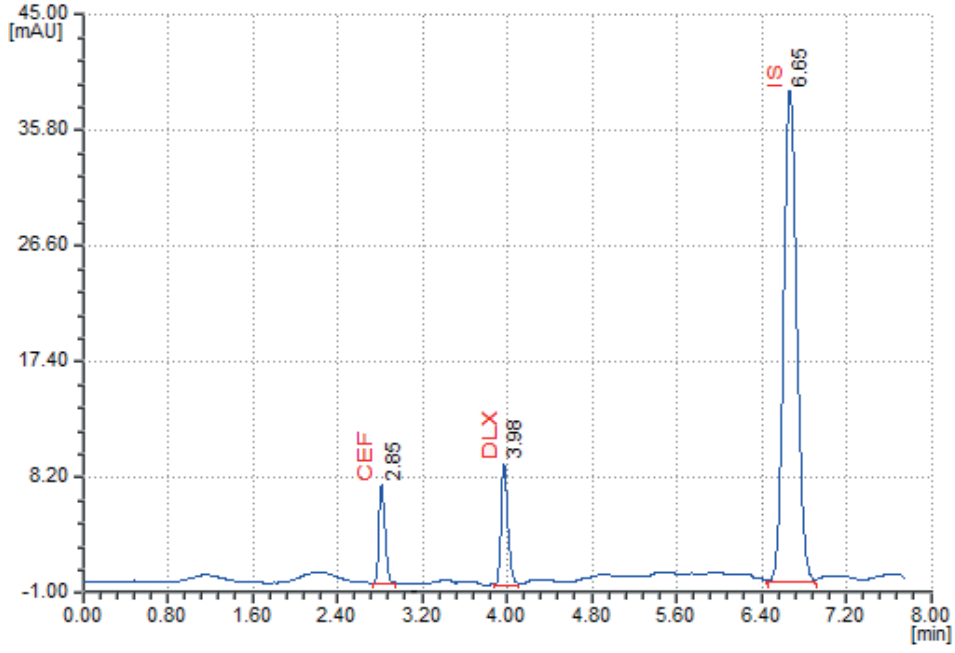

Figure 1. Chromatogram of 1 µg mL^{-1} of CFX and DLX with IS

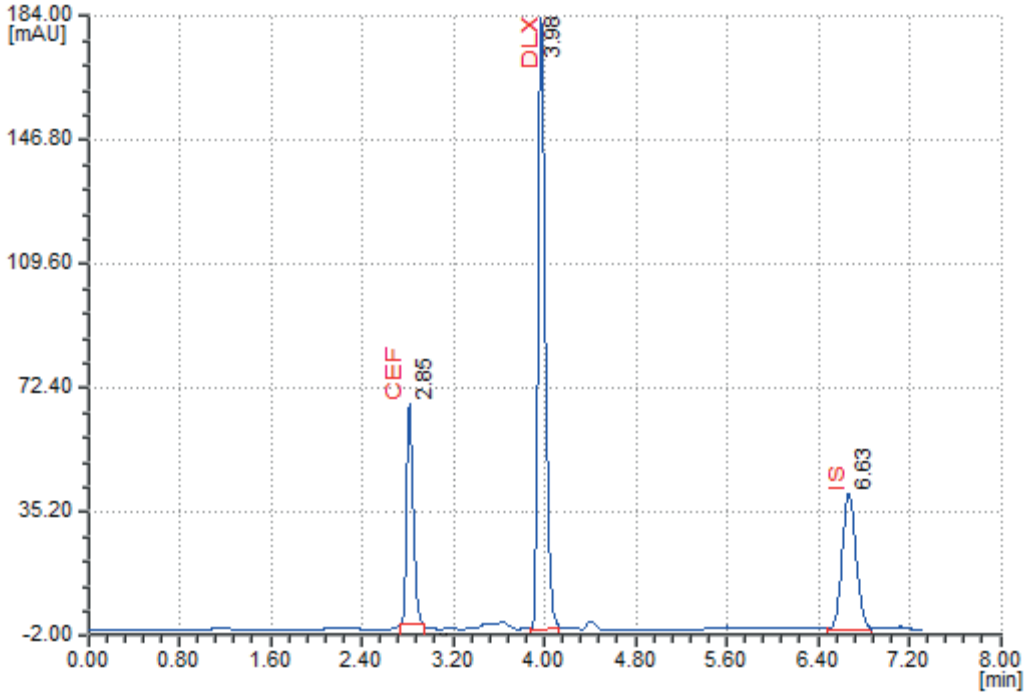

Figure 2. Chromatogram of cefixime (5 µg mL^{-1}), dicloxacillin (25 µg mL^{-1}) and IS from tablet

Table 1. System suitability test parameter for CFX and DLX having concentration 10 µg mL^{-1}

Ob No.	Retention Time		Tailing Factor		Theoretical Plate		Asymmetry	
	CFX	DLX	CFX	DLX	CFX	DLX	CFX	DLX
1	2.83	3.94	0.5267	0.7523	5025	4092	0.7535	0.8922
2	2.84	3.94	0.559	0.7025	5275	3989	0.6574	0.9135
3	2.85	3.95	0.5712	0.7398	5180	3895	0.7555	0.9567
4	2.83	3.93	0.5298	0.7085	5495	4089	0.7035	0.9812
5	2.83	3.95	0.5138	0.7544	5278	4175	0.698	0.8545
6	2.83	3.95	0.557	0.7598	5396	4012	0.7111	0.9855
Mean	2.8350	3.9433	0.5429	0.7362	5274.83	4042.00	0.7131	0.9306
S.D	0.0083	0.0081	0.0225	0.0247	164.0580	97.6893	0.0370	0.0525
%RSD	0.2951	0.2069	4.5586	3.3617	3.1102	2.4168	5.1923	5.6479

Table 2. Linear regression analysis of calibration curves (n = 3)

Drug	Linearity Range (µg mL^{-1})	Intercept	Slope	Correlation Coefficient (r^2)	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
CFX	1-25	262.5317	24424.7936	0.9998	0.025	0.321
DLX	1-25	1405.3091	28435.7217	0.9999	0.021	0.211

Accuracy (% Recovery)

The proposed method afforded a recovery of 99.99–101.36% after spiking the additional standard drugs solution to the previously analyzed test solutions. The percentage recoveries for CFX and DLX were found in the range of 99.99–101.36% and 100.16 to 100.58% respectively. The values of the recovery (%) and %RSD are shown in Table 3, which indicated the accuracy of the proposed method.

Precision

Intra-day precision of the method ranged from 0.2516 to 1.4603 %RSD for CFX and DLX. Inter-days precision of the method was found to be 0.2823 to 3.3567 %RSD for CFX and DLX, which indicate that the developed method is precise (Table 4). The low values of the RSD (%) indicate the repeatability of the proposed method.

LOD and LOQ

The LODs for CFX and CLX were found to be 0.025 and 0.021 µg mL^{-1}, while the LOQs for CFX and CLX were 0.321 and 0.211 µg mL^{-1}, respectively (Table 2).

Robustness

The deliberate changes in the method have not been much affected the peak tailing, theoretical plates and the percent assay of CFX and DLX by changing the composition of the mobile phase, flow rate and pH of phosphate buffer. The robustness study results are presented in Table 5.

Analysis of Marketed Formulations

The prescribed validated RP-HPLC method was successfully applied for simultaneous determination of CEF and DLX in the marketed formulation. There was no interaction between the CFX, DLX and the other excipients present in the tablet. The CFX and DLX content were found to be 99.50% and 100.70% with %RSD of 1.1723 and 1.7051, respectively (Table 6). It may, therefore, be inferred that the degradation of the CFX and DLX had not occurred in the tablet formulation that was analyzed by this method.

Table 3. Accuracy (% Recovery) determined with developed method (n = 3)

Excess drug added to analyte, %	Theoretical Content (µg mL^{-1})		Conc. Found (µg mL^{-1}) (mean ±SD)		%Recovery (mean ±SD)		%RSD	
	CFX	DLX	CFX	DLX	CFX	DLX	CFX	DLX
0	2.00	5.00	1.9998 ±0.0380	5.0133 ±0.0674	99.99 ±1.9021	100.26 ±1.3485	1.9022	1.3449
50	3.00	7.50	3.0408 ±0.0608	7.5441 ±0.0500	101.36 ±2.0286	100.58 ±0.6676	2.0013	0.6636
100	4.00	10.00	4.0028 ±0.0493	10.0368 ±0.0633	100.07 ±1.2343	100.36 ±0.6331	1.2334	0.6308
150	5.00	12.50	5.0228 ±0.0595	12.5207 ±0.0381	100.45 ±1.1900	100.16 ±0.3054	1.1846	0.3049

Table 4. Inter-day and Intra-days precision of CFX & DLX standards

Theoretical concentration (µg mL^{-1})	CFX				DLX			
	Intra-day measured concentration		Inter-days measured concentration[b]		Intra-day measured concentration		Inter-days measured concentration[b]	
	(Mean[a] ± S.D)	RSD%	(Mean[a] ± S.D)	RSD%	(Mean[a] ± S.D)	RSD%	(Mean[a] ± S.D)	RSD%
1	0.9975 ±0.0143	1.4384	0.9950 ±0.0130	1.3109	1.0038 ±0.0146	1.4603	1.0063 ±0.0337	3.3567
15	15.0227 ±0.1333	0.8876	15.0160 ±0.0517	0.3446	15.0186 ±0.0489	0.3255	14.9988 ±0.0636	0.4243
25	25.0542 ±0.0769	0.3071	25.0850 ±0.0710	0.2833	25.0422 ±0.0630	0.2516	25.0454 ±0.0707	0.2823

[a] Mean values represent six different CFX & DLX standards for each concentration
[b] Inter-days reproducibility was determined from six different runs for three consecutive day

Table 5. Robustness study

Variation	Chromatographic Parameter					
	Tailing Factor		Theoretical Plate		%Assay	
	CFX	CLX	CFX	CLX	CFX	CLX
47% of Phosphate buffer in Mobile Phase	0.5271	0.7390	5143.01	4155.38	99.91	100.11
43% of Phosphate buffer in Mobile Phase	0.5532	0.7141	5289.00	4275.67	101.28	99.99
Flow Rate at 0.9 mL min^{-1}	0.6043	0.7854	5578.57	4246.78	98.90	99.64
Flow Rate at 1.1 mL min^{-1}	0.5032	0.7071	5467.36	4244.54	101.43	99.05
pH of mobile phase at 5.3	0.5445	0.7445	5287.17	4131.45	100.05	101.67
pH of mobile phase at 5.5	0.5322	0.7323	5378.99	4094.05	98.45	99.59

Table 6. Analysis of tablet sample having concentration of CFX 10 µg mL^{-1} and DLX 25 µg mL^{-1}

Sr. No.	Total amount recovered (µg mL^{-1})		% Label claim	
	CFX	DLX	CFX	DLX
1	10.0383	25.5764	100.38	102.30
2	9.8045	25.0451	98.04	100.18
3	10.1090	24.9470	101.09	99.78
4	9.8564	24.4826	98.56	97.93
5	9.9954	25.5240	99.95	102.09
6	9.8956	25.4752	98.95	101.90
Mean	9.9498	25.1750	99.50	100.70
SD	0.1166	0.4292	1.1664	1.7170
%RSD	1.1723	1.7051	1.1723	1.7051

CONCLUSION

The proposed RP-HPLC method is accurate, precise, rapid, robust, sensitive and selective. The prescribed method adapted the use of the economical and easily available mobile phase, UV detector, and easy extraction procedures. Washing of the column with same mobile phase makes it an excellent method for the quantification of CFX and DLX in bulk drugs and the pharmaceutical dosage form. The results also suggest non-interference of formulation excipients in the estimation. The developed method can be used for the routine analysis of both the drugs from bulk and different formulations and could help in Therapeutic Drug Monitoring (TDM) and bioavailability studies.

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