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Development and Validation of Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Levofloxacin and Cefodoxime in Bulk and Their Combined Dosage Form.

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ABSTRACT

A simple, specific, and precise stability indicating reverse phase high performance liquid chromatography method was developed and validated as per the ICH guidelines for the simultaneous determination of Levofloxacin and Cefpodoxime in bulk and combined dosage forms. The quantification was carried out by using Inspire C_{18} (4.6*250mm, 5µm) column at 30^0c with Acetonitrile: Phosphate Buffer pH 4.0 in ratio of 60:40% V/V as mobile phase, pH 4.0 adjusted by using 0.1M ortho phosphoric acid. The flow rate is 1 mL/min and the estimation was carried out by using PDA detector at 275 nm. The retention time of LFX and CFDX were 2.227 and 3.821 minutes respectively. The linearity was observed from 75-125µg/mL with correlation coefficient 0.9997 for Levofloxacin and 60-100 µg/mL with correlation coefficient 0.9995 for Cefpodoxime. The LOD and LOQ of Levofloxacin and Cefpodoxime were found to be 0.035 & 0.12µg/mL and 0.080 & 0.280µg/mL respectively and the statistics data for the LFX and CFDX were concluded that the method was found to be simple, reliable, selective, reproducible and accurate. The method was successfully used for quality control analysis of Levofloxacin and Cefpodoxime.

Keywords: Levofloxacin (LFX), Cefpodoxime (CFDX), RP-HPLC, Stability, and Validation.

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INTRODUCTION

Levofloxacin is [(S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methy-l-piperazinyl)-7-oxo-7H-pyrido [l, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid [1,2] an oral broad-spectrum fluoroquinolone antibacterial agent and its molecular formula is $C_{18}H_{20}FN_3O_4$. It has a broad spectrum of activity against gram-positive and gram-negative bacteria Such as like chlamydia, mycoplasma and legionella [3] and it is used to treat certain bacterial infections like bacterial conjunctivitis, chronic bronchitis, sinusitis, pneumonia, abdominal infections and urinary tract infections. While compared to earlier fluoroquinoline antibiotics levofloxacin shows greater activity for gram-positive bacteria and lesser activity for gram- negative bacteria. Cefpodoxime is (6R, 7R)-7-{[(2Z)-2-(2-amino-1, 3-thiazol-4-yl)-2-methoxyimino-acetyl] amino}-3-(methoxymethyl)-8-oxo-5-thialazabicyclo [4.2.0]-Oct-2-ene-2-carboxylic acid [4, 5] and its molecular formula is $C_{21}H_{27}N_5O_9S_2$. Cefpodoxime Proxetil is a prodrug and its active metabolite is Cefpodoxime. It inhibits the cell wall synthesis by inhibiting final transpeptidation step of peptidoglycan synthesis in cell walls [6]. It is third generation cephalosporin oral antibacterial agent and active against gram-positive and gram-negative bacteria [7]. Levofloxacin and Cefpodoxime is a one of the newer combination of tablet dosage form which is used to treat the bacterial infections. Chemical structure of Levofloxacin and Cefpodoxime are shown in Figure No. 1 & 2 respectively.

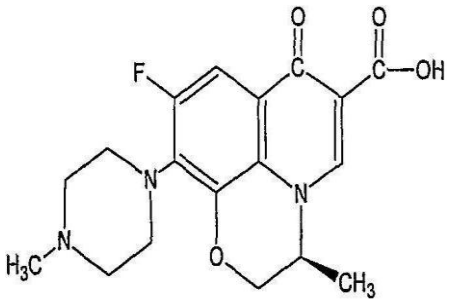

Figure 1: Levofloxacin

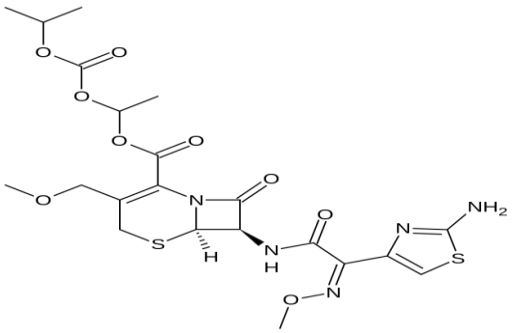

Figure 2: Cefpodoxime

MATERIALS AND METHODS

Materials

Levofloxacin and Cefpodoxime pure drugs were obtained as a gift sample from Cipla pharmaceuticals Ltd, Mumbai, India. HPLC grade Acetonitrile and water [filtered through 0.2µ filters] were purchased from Merck, Mumbai, India. Potassium dihydrogen phosphate and ortho phosphoric acid were purchased from Rankem, RFCL limited, New Delhi, India.

Preparation of Solutions

Stock and Standard solution

The stock solution prepared from pure drugs of 25mg of Levofloxacin and 20mg of Cefpodoxime were taken in 100mL volumetric flask and dissolved in 10mL of HPLC grade methanol, and diluted up to the mark with mobile phase.

The standard solution prepared from 4mL of stock solution was taken in 10mL volumetric flask and diluted up to the mark with mobile phase to get a concentration of 100µg/mL of Levofloxacin and 80µg/mL of Cefpodoxime.

Phosphate Buffer pH 4.0

Dissolve 6.8g of potassium di-hydrogen phosphate in 1000mL of HPLC grade water (filtered through 0.2µ filters) and degassed. Adjust the pH to 4.0 by 0.1M ortho phosphoric acid.

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Sample solution

20 tablets (Oridex-LV) of Levofloxacin and Cefpodoxime were powdered and an amount of the powder equivalent to 25mg of Levofloxacin and 20 mg of Cefpodoxime was accurately weighed and transferred to the 100mL volumetric flask, made up to the volume with mobile phase. The solution was placed in an ultrasonicator for 30 minutes and filtered through a 25 mm, 0.45 µm nylon syringe filter. 4mL of Levofloxacin and 5mL of Cefpodoxime solution was taken and diluted to 10mL by using a mobile phase to get a final concentration of 100µg/mL. Five replicate sample solutions were prepared in similar manner.

HPLC Instrumentation and Conditions

Instrumentation

Waters HPLC system consisting of WATERS 2695 separation module, an inbuilt auto sampler, column oven and WATERS 2996 (PDA) detector was employed for throughout the analysis. Chromatography was performed on a Inspire C_{18} column. A sonerex sonicator was used for sonication and the data was acquired by using the EM Power^2 software.

Optimized chromatographic conditions:

Table 1: Instrumentation and Optimized chromatographic conditions for proposed method

S. No	Instrumentation	Optimized Chromatographic Conditions
1	HPLC	Waters: 2695 Separation Module
2	Detector	Waters: 2996 PDA
3	Column	Inspire C_{18} (4.6*250mm, 5µm)
4	Column temperature	30^0C
5	Flow rate	1 mL/min
6	Injection volume	20µL
7	Wavelength	275 nm
8	Run time	8 minutes
9	Mobile phase composition	ACN: Phosphate Buffer in ratio of 60:40% V/V

Chromatography was performed on a Inspire C_{18} column using mobile phase containing mixture of Acetonitrile: Phosphate Buffer pH 4.0 in ratio of 60:40% V/V. The mobile phase was filtered through membrane filter (0.45 µm), and vacuum degassed by sonication prior to use. The pump pressure and run time was maintained at 1500-2500 psi and 8 minutes respectively. Chromatography was performed at 30^0C with flow rate at 1 mL/min and detection was carried out at 275 nm. Instrumentation and optimized chromatographic conditions for proposed method details are shown in Table No 1.

RESULTS AND DISCUSSION

Validation study of Levofloxacin and Cefpodoxime

The Method validation was performed as per ICH guidelines for the simultaneous estimation of Levofloxacin and Cefpodoxime in bulk and combined dosage form. The method was validated with respect to parameters including accuracy, precision, linearity, robustness, specificity, system suitability, LOD and LOQ [8].

Assay of Levofloxacin and Cefpodoxime

The developed method was applied to the assay of Levofloxacin and Cefpodoxime in combined dosage forms. The drug content was estimated with an average of six determinations, and results were given in Table No 2. The results were similar to the labeled claim of market formulations. The standard and sample chromatograms of Levofloxacin and Cefpodoxime were shown in Figure No 3 and 4 respectively.

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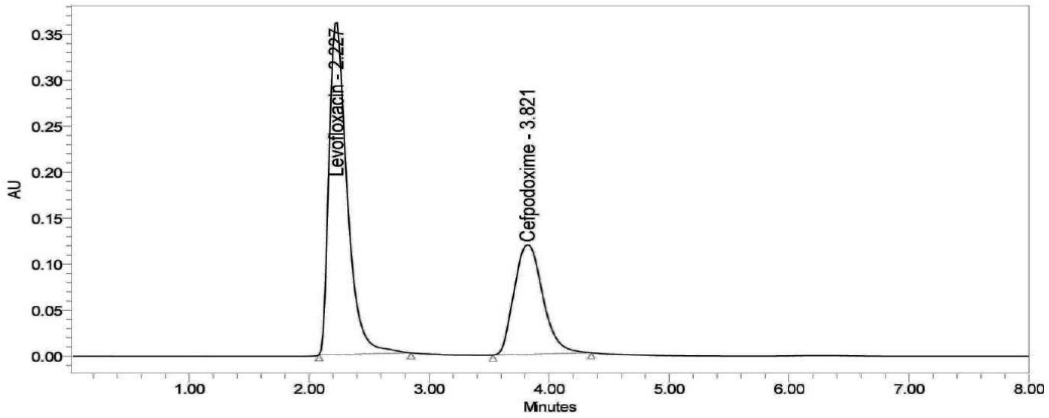
Table 2: Assay results of Levofloxacin and Cefpodoxime formulations

S. No	Formulations		Labeled Amount(mg)	Amount Found(mg)±S.D*	%Assay ±RSD
1	Oridex-LV Tablets	Levofloxacin	250	249.87±0.271	99.94±0.17
2		Cefpodoxime	200	199.77±0.127	99.88±0.57



	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Levofloxacin	2.227	3798624	366956	3297	1.55	
2	Cefpodoxime	3.821	1911585	117511	3421	1.26	4.54

Figure 3: RP-HPLC Chromatogram of Levofloxacin and Cefpodoxime

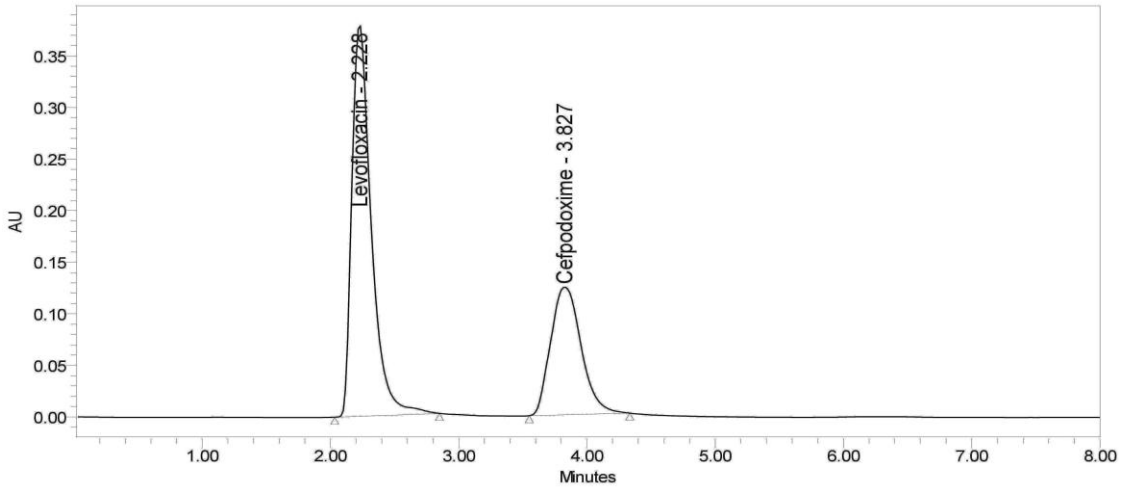


Figure 4: RP-HPLC chromatogram of Levofloxacin and Cefpodoxime formulation (Tablets)

Specificity

The specificity of the proposed method was established by injecting the placebo and mobile phase solution in triplicate and the chromatograms were recorded. Comparison of chromatograms revealed that there were no interactions between the placebo and sample peaks. Finally, it was indicated that the method was specific.

Accuracy

The accuracy was determined by calculating the recovery of Levofloxacin and Cefpodoxime at 50, 100, & 150% and they were added to pre quantified sample solution. The recovery studies were carried out in the dosage form in triplicate each in the presence of placebo. The mean percentage recovery of LFX and CFDX at

ISSN: 0975-8585

each level was not less than 99%, and not more than 102%. The percentage recovery of Levofloxacin and Cefpodoxime was found to be in the range of 99 to 101%. The results are shown in the Table No 3 and 4.

Table 3: Recovery data for the proposed RP-HPLC method for LFX

S. No	Concentration level (%)	Amount added (µg/mL)	Amount found (µg/mL)	Area obtained	Mean %Recovery ± S.D*	%RSD*
1	50	5.14	5.12	5719583	99.74±0.404	0.406
			5.15	5768741		
			5.11	5830173		
2	100	10.40	10.47	7785920	100.22±0.400	0.399
			10.39	7771667		
			10.41	7808624		
3	150	15	14.97	9497411	99.91±0.138	0.138
			15.01	9475145		
			14.98	9497133		

Table 4: Recovery data for the proposed RP-HPLC method for CFDX

S. No	Concentration level (%)	Amount added (µg/mL)	Amount found (µg/mL)	Area obtained	Mean %Recovery ± S.D*	%RSD*
1	50	5.1	5.01	2919845	98.49±0.816	0.828
			4.99	3046912		
			5.07	2979311		
2	100	10.49	10.52	4105896	100.09±0.252	0.251
			10.47	4020585		
			10.51	4106719		
3	150	15.18	15.17	5081374	99.84±0.402	0.403
			15.09	5007123		
			15.21	5012641		

***S.D & %RSD** is Standard Deviation and percentage of Relative Standard Deviation

Precision

Precision should be investigated by using authentic, and homogeneous samples. The Precision of this method was expressed as S.D and %RSD of series of repeated measurements. Precision of LFX and CFDX determination by proposed method were ascertained by repeated analysis of homogeneous samples of Levofloxacin and Cefpodoxime standard solutions in the intraday under the similar conditions. The system and method precision results were shown in Table No 5 and 6.

Table 5: System Precision results of the proposed RP-HPLC method

S. No	Injections	LEVOFLOXACIN		CEFPODOXIME	
		Retention Time	Peak Area	Retention Time	Peak Area
1	1	2.228	3993628	3.827	2044176
2	2	2.228	3993401	3.829	2055630
3	3	2.230	4003269	3.830	2044670
4	4	2.233	4006984	3.832	2071760
5	5	2.229	4011440	3.829	2093409
6	6	2.229	4025012	3.831	2041166
7	**MEAN**	**2.229**	**4005622**	**3.829**	**2058468**
8	**SD**	**0.0018**	**11919**	**0.0017**	**20491**
9	**%RSD**	**0.083**	**0.297**	**0.045**	**0.995**

ISSN: 0975-8585

Table 6: Method Precision results of the proposed RP-HPLC method

S. No	Injections	LEVOFLOXACIN		CEFPODOXIME	
		Retention Time	Peak Area	Retention Time	Peak Area
1	1	2.227	3737469	3.822	1907172
2	2	2.230	3757364	3.827	1936100
3	3	2.230	3769693	3.826	1981277
4	4	2.228	3791666	3.826	1917403
5	5	2.228	3799467	3.827	1939966
6	6	2.228	3781073	3.826	1967353
7	**MEAN**	**2.228**	**3772788**	**3.825**	**1941545**
8	**SD**	**0.0012**	**22942**	**0.0018**	**28429**
9	**%RSD**	**0.0549**	**0.608**	**0.048**	**1.464**

Linearity

Linearity of the proposed method was established by using series of standard solutions of Levofloxacin and Cefpodoxime, and these studies are repeated in triplicate with different stock solutions. The curve obtained by concentration on x-axis and peak area on y-axis against showed linearity in the concentration range of 75 to 125µg/mL for Levofloxacin and 60-100µg/mL for Cefpodoxime and linearity graph is shown in Graph No 1 and 2. The regression equation and correlation coefficient of Levofloxacin and Cefpodoxime were found to be Y=36744x+8445 & 0.9997 and Y=24730x+1348 & 0.9995 respectively. The Linearity and statistical analysis of data are shown in Table No 7 and 8.

Table 7: Linearity and Statistical analysis data for Levofloxacin

S. No	Concentration (µg/mL)	Area	Average Area	Statistical Analysis		
				Slope	Y-Intercept	Correlation Coefficient
1	75	2780635	3680910	36744	8445	**0.9997**
2	87.5	3224064				
3	100	3693582				
4	112	4120221				
5	125	4586047				

Table 8: Linearity and Statistical analysis data for Cefpodoxime

S. No	Concentration (µg/mL)	Area	Average Area	Statistical Analysis		
				Slope	Y-Intercept	Correlation Coefficient
1	60	1487588	1980055	24730	1348	**0.9995**
2	70	1722589				
3	80	1990004				
4	90	2240164				
5	100	2459928				

Robustness

The robustness was evaluated by the analysis of Levofloxacin and Cefpodoxime under different experimental conditions such as slight changes in chromatographic conditions like change of flow rate (±0.2 mL/min), temperature (±5^{0}C), and mobile phase composition (± 5%). It was distinguished that there were no changes in the chromatograms, and the parameters were within the limits, which indicates that the method was robust and suitable for routine use. The complete results are shown in Table No 9 & 10, and the method is having good system suitability.

ISSN: 0975-8585

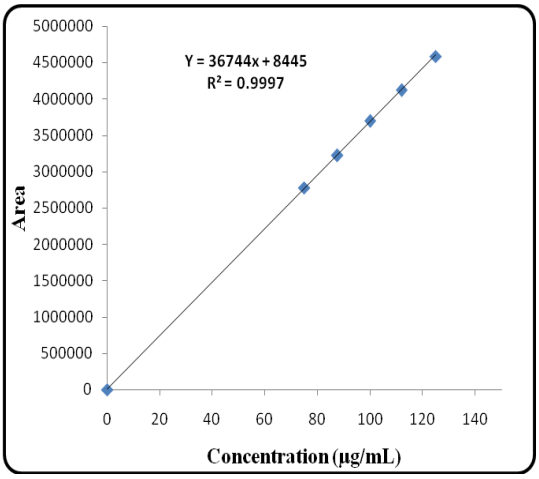
Table 9: Robustness results of the proposed RP-HPLC method for Levofloxacin

S. No	Parameters			Peak Area	RT*	USP	
	Optimized		Used			Plate Count	Tailing Factor
1	Flow rate (±0.2)	**1 mL/min**	0.8	4643248	2.773	3140	1.43
			1.2	3305314	1.871	3208	1.52
2	Temperature (±5^{0}c)	**30^{0}c**	25	3462548	2.79	2789	1.97
			35	4575477	1.87	3107	1.77
3	Mobile phase composition (± 5%)	**60:40**	65:35	3811025	2.176	2365	1.39
			55:45	4243003	2.322	3748	1.86

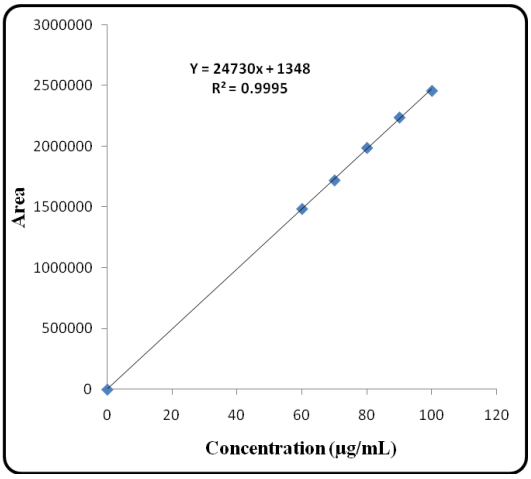
Table 10: Robustness results of the proposed RP-HPLC method for Cefpodoxime

S. No	Parameters			Peak Area	RT*	USP		Resolution
	Optimized		Used			Plate Count	Tailing Factor	
1	Flow rate (±0.2)	**1 mL/min**	0.8	2326895	4.793	3418	1.08	4.72
			1.2	1751321	3.207	3435	1.22	4.66
2	Temperature (±5^{0}c)	**30^{0}c**	25	1877547	2.972	2987	1.87	4.29
			35	229871	3.071	3781	1.98	4.78
3	Mobile phase composition (± 5%)	**60:40**	65:35	923233	4.918	2254	1.15	4.86
			55:45	2207268	3.256	3678	1.29	3.49

*** RT** is Retention Time



Graph 1:Linearity Graph of Levofloxacin



Graph 1:Linearity Graph of Cefpodoxime

Limit of Detection

The limit of detection (LOD) has established the minimum concentration at which the analyte can be reliably detected. LOD is determined by the signal to noise ratio and generally acceptable detection limit ratio is 3:1. It was found to be 0.035µg/mL for Levofloxacin and 0.080 µg/mL for Cefpodoxime respectively.

Limit of Quantification

The limit of quantification (LOQ) has established the minimum concentration at which the analyte can be reliably quantified. LOQ is determined by the signal to noise ratio and a typical signal to noise ratio is 10:1 is acceptable for estimating the quantification limit. It was found to be 0.12 µg/mL for Levofloxacin and 0.280 µg/mL for Cefpodoxime respectively.

ISSN: 0975-8585

System suitability

This test was conducted on freshly prepared Levofloxacin and Cefpodoxime standard solution was used for the evaluation of the system suitability parameters such as retention time, area, USP tailing and theoretical plates, limit of detection and limit of quantification. Five replicate injections for a system suitability test were injected into the chromatographic system. Finally the proposed method is having good system suitability and its parameters are shown in Table No 11.

FORCED DEGRADATION STUDY

Forced degradation studies were conducted to evaluate the stability and specificity of the method. The acceptable limit for consideration in the present study is between 5 to 20% for chromatographic assays [9, 10]. The specificity of the developed method was evaluated by using different ICH prescribed stress conditions like acidic, basic, oxidative, and thermal.

Acidic Degradation

These studies can be performed by taking 10 mL stock solution of Levofloxacin and Cefpodoxime, each in separate 50 mL volumetric flask. 10 mL of 5N HCL was added to the stock solution and these solutions were kept at reflux for 4 hours. Finally this solution was neutralized with 5 N NaOH.

Alkali Degradation

These studies can be performed by taking 10 mL stock solution of Levofloxacin and Cefpodoxime, each in separate 50 mL volumetric flask. 10 mL of 5 N NaOH was added to the stock solution and these solutions were kept at reflux for 4 hours. Finally this solution was neutralized with 5N HCL.

Oxidative Degradation

These studies can be performed by taking 10 mL stock solution of Levofloxacin and Cefpodoxime, each in separate 50 mL volumetric flask. 10 mL of 3% hydrogen peroxide added to each flask. These mixtures were kept for up to 3 days in the dark.

Thermal Degradation

These studies can be performed by taking 10 mL stock solution of Levofloxacin and Cefpodoxime, each in separate 50 mL volumetric flask, then sample solution were heated to 80^0c for 15-60 minutes.

Finally forced degradation studies of Levofloxacin and Cefpodoxime concluded that purity of angle less than purity of threshold and forced degradation chromatogram were shown in Figure No 5 to 8. All the Degradation summary results were shown in Table No: 12

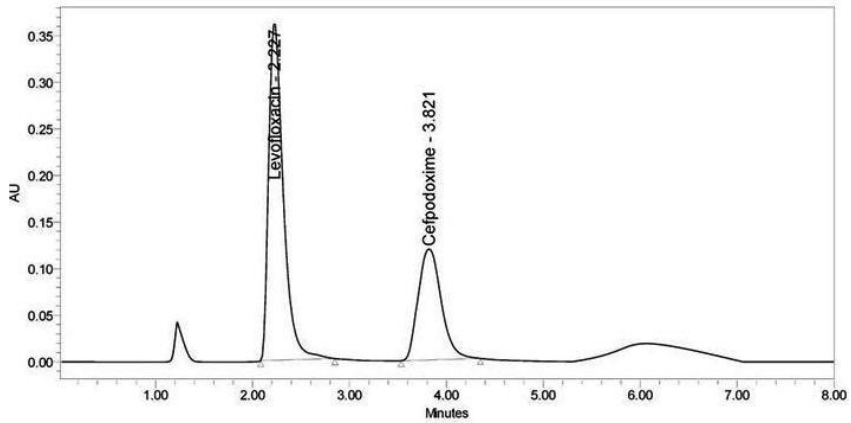


Figure 5: Chromatogram of Levofloxacin and Cefpodoxime for Acidic Degradation

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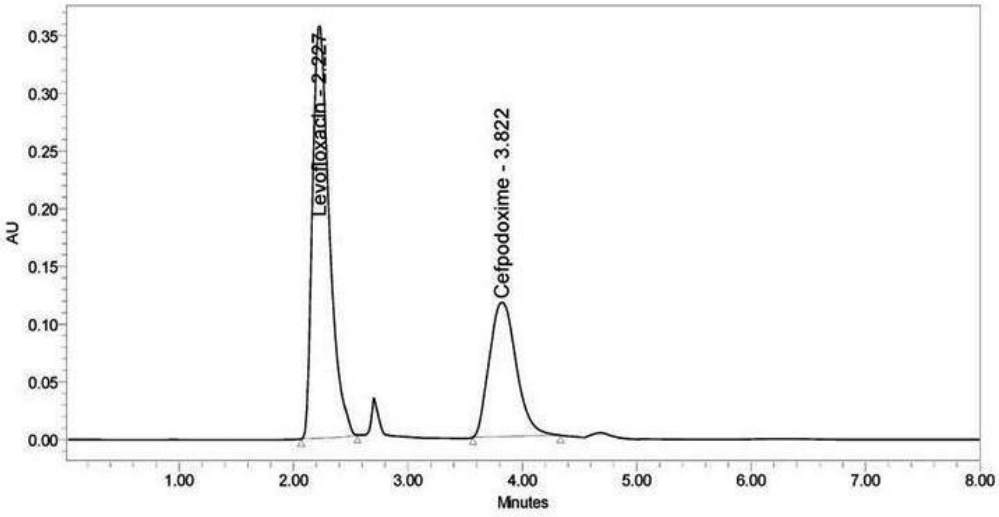

Figure 6: Chromatogram of Levofloxacin and Cefpodoxime for Alkali Degradation

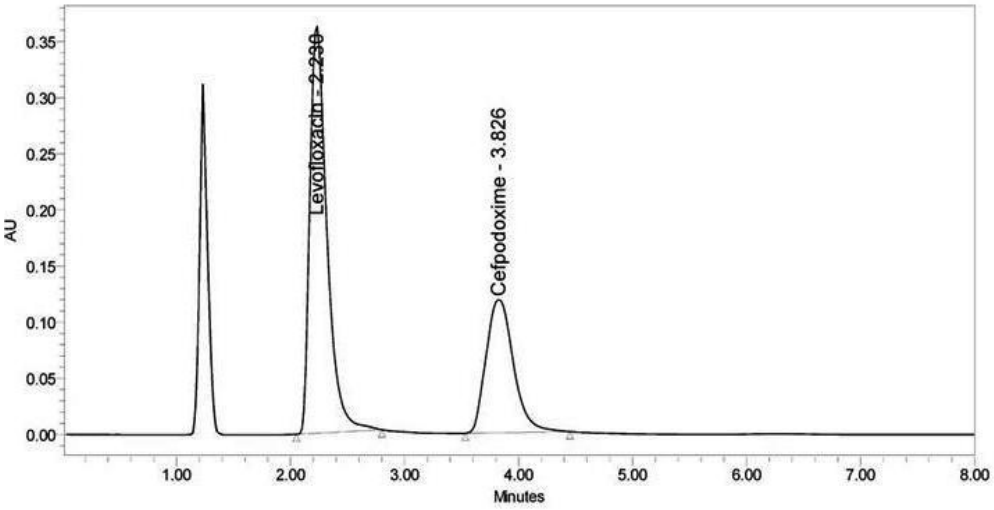

Figure 7: Chromatogram of Levofloxacin and Cefpodoxime for Oxidative Degradation

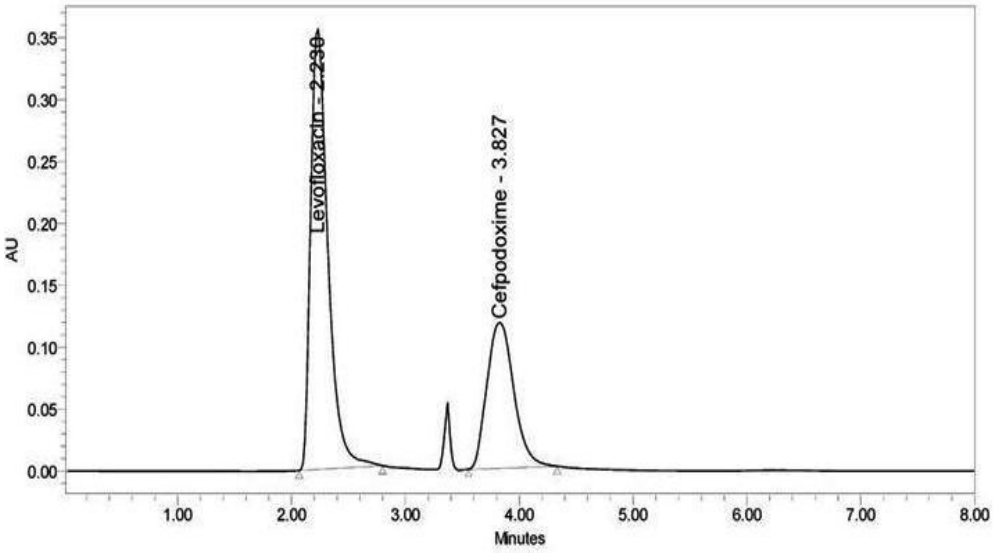

Figure 8: Chromatogram of Levofloxacin and Cefpodoxime for Thermal Degradation

ISSN: 0975-8585

CONCLUSION

A stability indicating RP-HPLC method for simultaneous estimation of Levofloxacin and Cefpodoxime in bulk and pharmaceutical dosage forms is established. The method is simple, accurate, linear, sensitive and reproducible as well as economical for the effective quantitative analysis of Levofloxacin and Cefpodoxime in bulk and combined dosage forms. The method was validated, and all the method validation parameters were tested and shown to produce satisfactory results. The method is free from interactions of the other ingredients and excipients used in the formulations. Finally concluded that the method is suitable for use in the routine quality control analysis of Levofloxacin and Cefpodoxime in active pharmaceutical ingredients and in pharmaceutical dosage forms.

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