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A NEW STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME AND LINEZOLID IN PHARMACEUTICAL DOSAGE FORM AND PLASMA SAMPLE

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ABSTRACT

A new stability indicating HPLC method was developed and validated for the determination of Cefixime and Linezolid in tablet dosage form and spiked plasma sample. The chromatographic separation was achieved on a X-Terra RP-18 (150mm x 4.6mm, 5µm) stationary phase maintained at a temperature of 30 ºC with a mobile phase combination of 1% Orthophosphoric acid and Methanol (60:40) at a flow rate of 1.0 mL min^{-1} and the detection was carried out by using UV detector at 250 nm. The total run time was 8 minutes. The retention time of Cefixime and Linezolid were found to be 3.815 min and 5.665 min respectively. Further forced degradation study of Cefixime and Linezolid has been carried out in various stress conditions. Both compounds were found to be sensitive to all accelerated conditions like acid, base, light, heat and oxidation. The performance of the method was validated according to the present ICH guidelines. Cefixime is found to be linear in the range of 100-300 µg mL^{-1} and Linezolid is found to be linear in the range of 300-900 µg mL^{-1} . The limit of detection and limit of quantification was found to be 0.6 µg mL^{-1} and 2.46 µg mL^{-1} for Cefixime and 2.01 µg mL^{-1} and 8.21 µg mL^{-1} for Linezolid. The proposed method was found to be simple, sensitive, accurate and can be applied for qualitative, quantitative and stability studies

Keywords: Cefixime, Linezolid, HPLC, Validation, Forced degradation study

INTRODUCTION

Cefixime, an antibiotic, is a third-generation cephalosporin like ceftriaxone and cefotaxime[1]. Chemically, it is known as (6R,7R)-7- [(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-[(carboxymethoxy) imino] acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid[2]. Like all β-lactam antibiotics, cefixime binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefixime interferes with an autolysin inhibitor[3].

Linezolid is a synthetic antibiotic, the first of the oxazolidinone class.Chemically, it is known as N-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl) phenyl [-2-oxo-1,3-oxazolidin-5-yl]methyl}acetamide. It selectively inhibits bacterial protein synthesis through binding to sites on the bacterial ribosome and prevents the formation of a functional 70S-initiation complex. Specifically, linezolid binds to a site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex, which is an essential component of the bacterial translation process [4].The structures of Cefixime and Linezolid were shown in Fig-1.

The literature survey revealed that there are only two HPLC methods[5-6] were developed for the estimation of Cefixime and Linezolid in combination dosage forms and also different HPLC methods were developed for the estimation of Cefixime in combination with Dicloxacillin[7], Cloxacillin[8].Other methods like zero order [9-10],

first order[11-12],second derivative[13] spectroscopic methods were available for the determination of Cefixime and Linezolid in pure and combined dosage forms.

There are no methods available for the determination of Cefixime and Linezolid in plasma samples. Hence there is a need to develop simple, precise, accurate, robust methods for their determination from pharmaceutical dosage form and plasma samples. The present study was aimed to develop a new stability indicating method for simultaneous estimation of Cefixime and Linezolid in combined pharmaceutical dosage form and also in plasma.

EXPERIMENTAL

Instrumentation: The Waters HPLC (Alliance) system comprised of autosampler, column oven and photo diode array detector was used for method development and method validation. The output signal was monitored and processed by using Empower software.

Materials: Cefixime and Linezolid bulk drugs were made available from Lara Drugs Pvt. Ltd, orthophosphoric acid, methanol were obtained from Merck. Commercially available Cefixime and Linezolid tablets were used for the dosage form analysis. All chemicals and reagent used were of HPLC grade, Milli-Q-water was used throughout the experiment. The pharmaceutical dosage form assayed in the study is Lizokef containing 200mg of Cefixime and 600mg of Linezolid.

Chromatographic conditions: The mobile phase used was mixture of buffer of 1% Orthophosphoric acid (*p*H-1.7) and Methanol in the ratio of 60:40 employing isocratic elution at a flow rate of 1.0 mL min^{-1}. The analytical column used was X-Terra RP-18 (150mmx4.6mm,5µm) at a temperature of 30°C .The detection was carried out at a wavelength of 250nm for a run time of 8 min.

Sample Preparation: The Standard and sample stock solutions were prepared in methanol and the working solutions were prepared in the mobile phase. They were injected into the HPLC system by using above mentioned chromatographic conditions.

Assay of Pharmaceutical Dosage form: Twenty tablets of Lizokefwere weighed to determine avarage tablet weight. Crushed the tablets and accurately weighed 1053 mg and transferred it into 100mL volumetric flask and methanol was added to extract Cefixime and Linezolid. From this 5 mL was taken and transferred it to 100 mL volumetric flask and made up the volume using mobile phase.

Extraction of drugs from Plasma: Serial dilutions of analytes were prepared in mobile phase and 1mL of each dilution were spiked into 100µL of plasma in a polypropylene centrifuge tubes. 0.5 mL of methanol was added and cyclomixed for 5 minute to extract the drugs. All the tubes were centrifuged for 30 minute at 3000 rpm. Supernatant were collected in another eppendorf tube and 20µL supernatant was injected into the analytical column.

Degradation Studies: Stress testing of the drug substance can help to identify the likely degradation products, stability of the molecule and also validate the stability and specificity of the analytical procedures. For degradation studies, solutions of drugs in methanol containing target concentration were mixed with acid, base, peroxide to undergo acidic, alkali, oxidative degradation. These samples were exposed to temperature, light and moisture to undergo thermal, photolytic and hydrolytic degradation. After the stress assays, the sample were analyzed in the above reported chromatographic conditions.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: At the beginning, the method development process was carried by using mobile phase of water, acetonitrile and buffers of various *p*Hon different columns like C8,C18, cyano and phenyl column. Optimization of mobile phase was performed based on resolution, tailing factor and peak area obtained for both Cefixime and Linezolid.

The mobile phase, 1% Orthophophoric acid (*p*H-1.7):methanol (60:40 v/v) was found to be satisfactory and gave two symmetric and well-resolved peaks for Cefixime and Linezolid. The retention time of Cefixime and Linezolid were found to be 3.815 and 5.665 respectively. The USP plate count and tailing factor were 11923, 11857 and 1.173, 1.023 for Cefixime and Linezolid respectively. The USP resolution between Cefixime and Linezolid was 8.288. The standard chromatogram was shown in Fig-2.

Validation of proposed method: The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[14-15].

Assay: The standard and sample solutions were prepared from formulation and plasma and can be injected into HPLC system.The % assay for Cefixime and Linezolid were calculated and they were shown in table-1 and the chromatograms were shown in Fig-3 and 4.

Linearity: Linearity test solutions of Cefixime and Linezolid were prepared from the stock solution at five different concentration levels from 50% to 150% of target concentration. The calibration curves were constructed by plotting peak areas versus its corresponding concentrations. The slope, Y-intercept and correlation coefficient of the calibration curve were calculated. The correlation coefficient was found to be 0.999 for both the drugs. The linearity range for Cefixime and Linezolid were found to be 100-300 µg mL^{-1} and 300-900 µg mL^{-1} respectively. The results were shown in Table-2 and the calibration curve for Cefixime and Linezolid were shown in Fig-5 and Fig-6.

Precision: Precision was evaluated by injecting 6 replicate injections of Cefixime and Linezolid of standard concentration under the same chromatographic conditions and calculated the % RSD. The %RSD indicates that the developed method is repeatable. The %RSD for assay of Cefixime and Linezolid were found to be 0.10 and 0.14 in formulation and 0.54 and 0.10 in plasma sample. The results were shown in Table-3 and 4.

Accuracy: In order to judge the quality and applicability of method the recovery analysis was performed at three levels like low, middle and high concentrations (50%, 100% and 150%) by standard addition method. The % recovery for Cefixime and Linezolid were calculated by injecting the samples and the results were shown in Table-5 and 6.

Robustness: Robustness as a measure of method capability to remain unaffected by small, but deliberate changes in chromatographic conditions was studied by testing influence of small changes in mobile phase *p*H (±0.2),organic phase composition(90% to 110%), column temperature (± 5^oC) and flow rate (± 0.2 mL min^{-1}).System suitability parameters like USP plate count, USP tailing and Resolution were checked and they found to be within the limits. The results were shown in Table-7.

LOD and LOQ: In accordance with International Conference on Harmonisation (ICH) recommendations, the approach based on the standard deviations (SD) of the response and the slope of the calibration plot was used for determinations of limit of detection and limit of quantification. The LOD and LOQ for Cefixime and Linezolid were found to be 0.60 µg mL^{-1} and 2.01 µg mL^{-1} and 2.46 µg $mL^{-1}$and 8.21 µg mL^{-1},respectively. It shows that method is sensitive for the determination of Cefixime and Linezolid and the results were shown in Table 8.

Degradation studies: The % assays for both the drugs were calculated from the assay, it was observed that the degradation of Cefixime and Linezolid in presence of light was more when compared with acid ,base, thermal and hydrolytic degradation. The effect of degradation on retention time of Cefixime and Linezold was evaluated, this indicates the stability indicating capacity of proposed method. The degradation chromatograms were shown in Fig-7,8 and 9 and the results were shown in Table 9.

CONCLUSION

In the present study describes development of new stability indicating simple, economic HPLC method. This developed and validated method was applied for estimation of Cefixime and Linezolid in pharmaceutical dosage form in accordance with the ICH parameters. The method gives good resolution between both the compounds with a short analysis time (8 minutes). The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of Cefixime and Linezolid in their combined dosage form.

Authors' statements: *Competing Interests*- The authors declare no conflict of interests

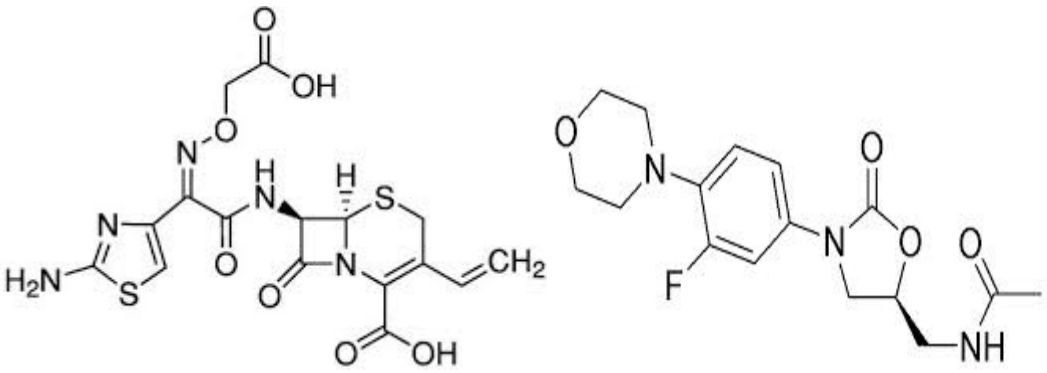

Fig.1. Structures of Cefixime and Linezolid

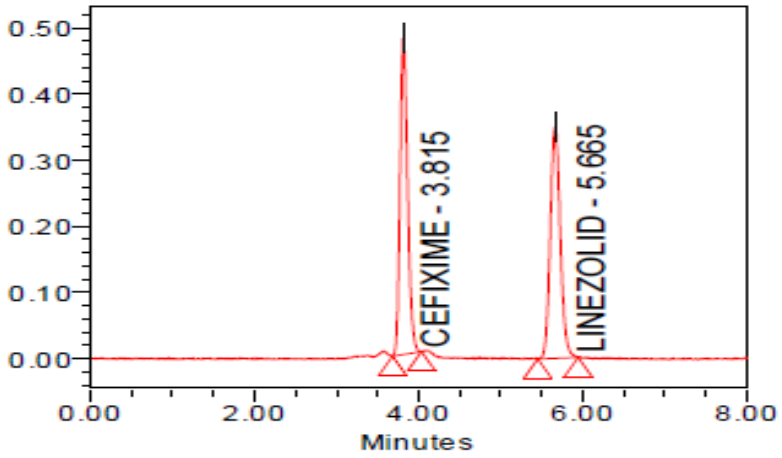

Fig.2. Standard chromatogram of Cefixime and Linezolid

Tab.1. Assay Results For Cefixime And Linezolid in Formulation and Plasma

	% Assayof Cefixime	% Assay Linezolid
Formulation	99.80	99.89
Plasma	99.16	99.50

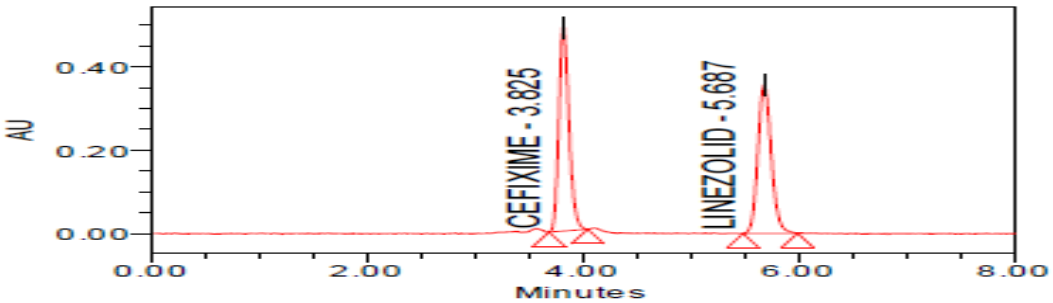

Fig.3. Sample (formulation) chromatogram of Cefixime and Linezolid

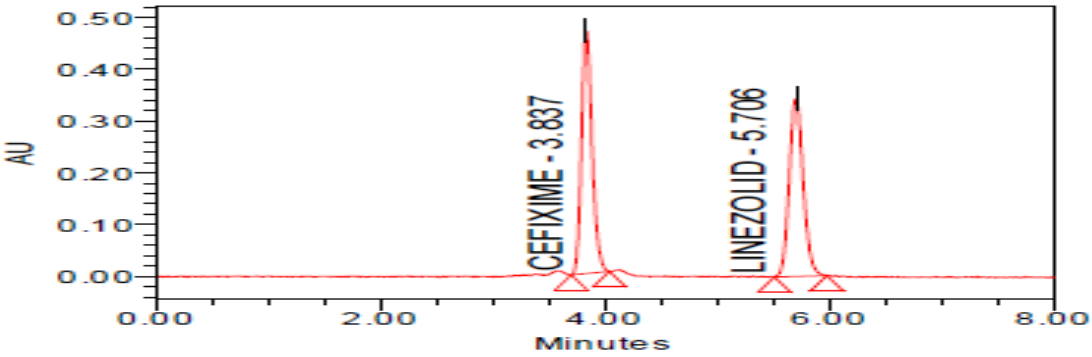

Fig.4. Sample (Plasma) chromatogram of Cefixime and Linezolid

Tab.2. Regression and statistical parameters

Parameter	Cefixime	Linezolid
Concentration Range (µg mL^{-1})	100-300	300-900
Correlation coefficient	0.999	0.999
Intercept	33168	25244
Slope	3571	812.2

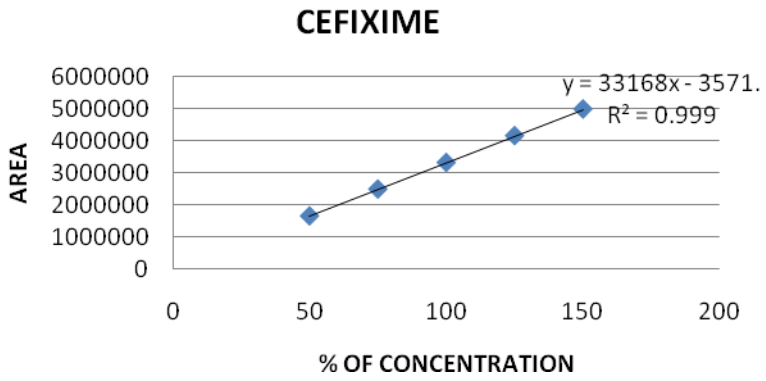

Fig.5. Calibration curve of Cefixime

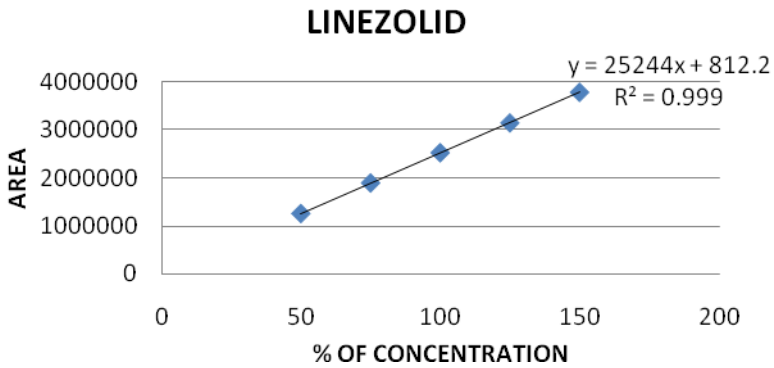

Fig.6. Calibration curve of Linezolid

Tab.3. Precision Results ForCefixime And Linezolid in Formulation

S.No	CEFIXIME		LINEZOLID	
	Area	% Assay	Area	% Assay
1	3311399	99.69	2527187	99.97
2	3317809	99.88	2529419	100.06
3	3310351	99.66	2527446	99.98
4	3318069	99.89	2523557	99.83
5	3315561	99.82	2520574	99.71
6	3316839	99.86	2522212	99.78
Average		99.80		99.89
Standard deviation		0.10		0.14
% RSD		0.10		0.14

Tab.4. Precision Results For Cefixime And Linezolid in plasma sample

S.No	CEFIXIME		LINEZOLID	
	Area	% Assay	Area	% Assay
1	3284522	98.88	2513627	99.44
2	3317453	99.87	2513620	99.44
3	3281099	98.78	2514608	99.48
4	3283032	98.84	2519029	99.65
5	3316029	99.83	2517697	99.60
6	3280453	98.76	2512416	99.39
Average		99.16		99.50
Standard deviation		0.54		0.10
% RSD		0.54		0.10

Tab.5. Accuracy Results for Cefixime and Linezolid in Formulation

Analyte	% Level	Nominal Value (µg mL^{-1})	Found (µg mL^{-1})	% Recovery	%RSD
Cefixime	50%	100	99.65	99.55	0.2
	100%	200	199.54	99.77	0.15
	150%	300	299.42	99.78	0.07
Linezolid	50%	300	299.84	99.85	0.19
	100%	600	599.63	99.94	0.07
	150%	900	898.52	99.8	0.03

Tab.6. Accuracy Results for Cefixime and Linezolid in Plasma samples

Analyte	% Level	Nominal Value (µg mL^{-1})	Found (µg mL^{-1})	% Recovery	%RSD
Cefixime	50%	99.10	97.84	98.74	0.21
	100%	198	197.05	99.52	0.48
	150%	297	293.64	98.84	0.05
Linezolid	50%	300	295.56	99.85	0.19
	100%	594	592.19	99.68	0.52
	150%	891.3	885.86	99.39	0.65

Tab.7. Robustness Results for Cefixime and Linezolid

	Cefixime			Linezolid		
	Retention time	**USP Plate count**	**USP Tailing**	**Retention time**	**USP Plate count**	**USP Tailing**
Flow 1	4.926	11378	1.153	6.692	10373	1.063
Temp 1	4.924	11021	1.167	6.681	9775	1.085
MP1	4.379	10549	1.15	5.944	8821	1.117
pH1	4.925	10210	1.158	6.713	9475	1.095
Flow 2	3.282	9086	1.149	6.756	7899	1.122
Temp 2	3.582	8906	1.18	6.732	7649	1.145
MP2	3.584	8783	1.16	6.628	7156	1.141
pH2	3.581	7901	1.156	5.862	7359	1.151

Flow 1- 0.8 mL min^{-1} , Flow 2- 1.2 mL min^{-1}, Mobile Phase composition 1- 360:640 (1% OPA : Methanol), Mobile Phase 2- 440:560 (1% OPA : Methanol),Temp 1-25ºC, Temp 2-35ºC, pH 1-1.5, *p*H 2-1.9

Tab.8. LOD and LOQ Results for Cefixime and Linezolid

	Cefixime		Linezolid	
	Conc (µg mL^{-1})	**S/N ratio**	**Conc(µgmL^{-1})**	**S/N ratio**
LOD	0.6	3.282	2.01	3.961
LOQ	2.46	10.204	8.21	10.951

Tab.9. Degradation Results ForCefixime And Linezolid

Type of Degradation	**Cefixime (% Assay)**	**Linezolid (% Assay)**
Acid	88	86
Base	80	82
Peroxide	78	82
Thermal	89	94
Photolytic	62	54
Hydrolytic	70	93

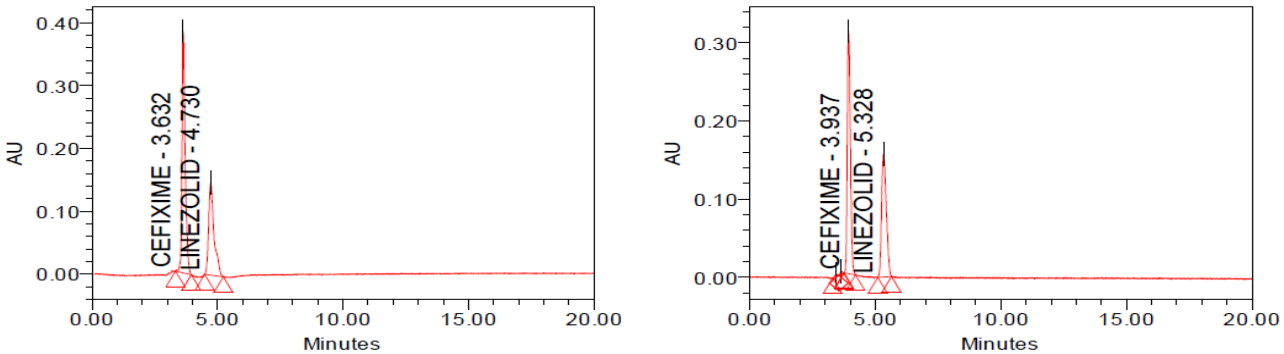

Fig.7. Degradation of Cefixime and Linezolid in presence of acid and base

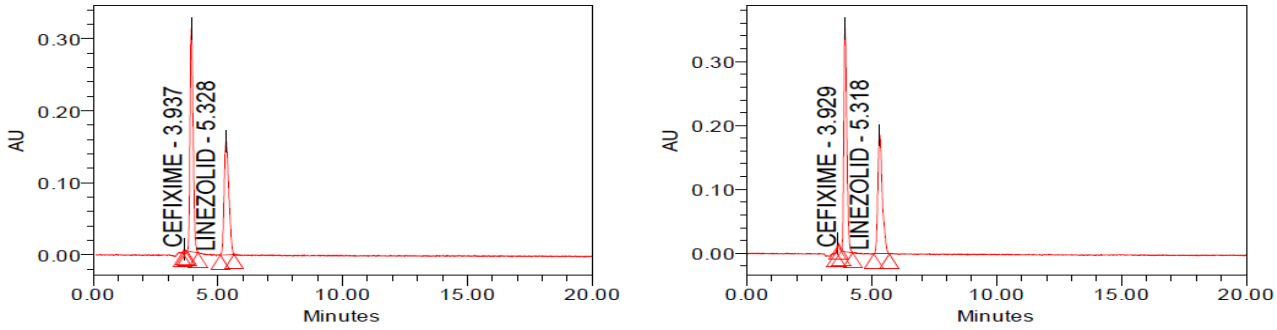

Fig.8. Degradation of Cefixime and Linezolid in presence of peroxide and water

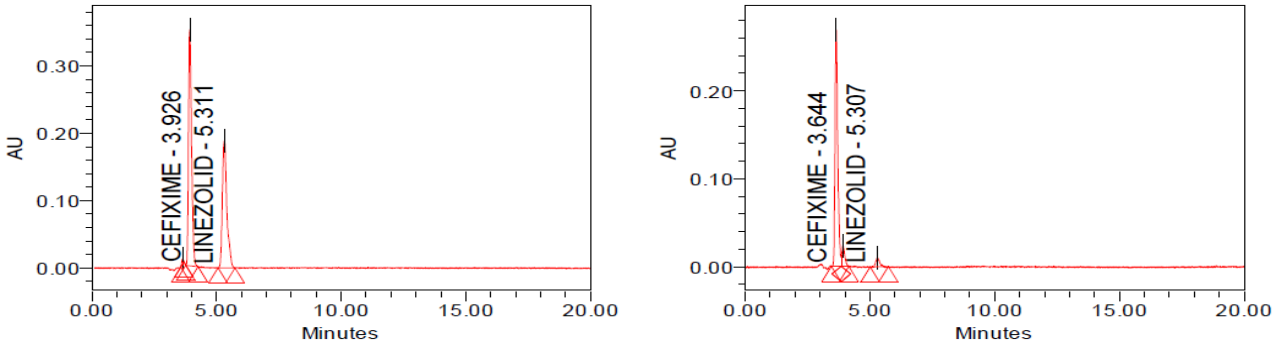

Fig.9. Degradation of Cefixime and Linezolid in presence of thermal and light

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