SIMULTANEOUS METHOD DEVELOPMENT AND VALIDATION OF CEFIXIME AND LACTOBACILLUS BY USING RP-HPLC.

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Cefixime and Lactobacillus in pharmaceutical dosage form. Chromatographic separation of Cefixime and Lactobacillus was achieved on Waters Alliance -2695, by using Symmetry shield RP 18, 150x4.6mm, 5 µm column and the mobile phase containing 3.0gm Octane-1-Sulphonic acid is dissolved in 1lt water adjust pH-2.5 with OPA **& ACN** in the ratio of **60:40% v/v**. The flow rate was 1.0 ml/min, detection was carried out by absorption at **282 nm** using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Cefixime and Lactobacillus were NLT 2000 and should not more than **2** respectively. % Relative standard deviation of peak areas of all measurements always less than **2.0.** The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Cefixime and Lactobacillus and study of its stability.

Key words: HPLC, Cefixime and Lactobacillus.

INTRODUCTION

Cefixime an anti-microbial [1] helpful to treat various bacterial diseases [2]. This incorporates Otitis media [3], strep throat, pneumonia [4], urinary tract contaminations, gonorrhoea and Lyme malady. For gonorrhoea regularly just a single measurements is required [5]. In the United States it is a second line treatment to ceftriaxone for gonorrhoea [6]. It is taken by mouth.

Basic symptoms incorporate diarrhoea [7], stomach agony, and sickness. Genuine symptoms may incorporate unfavourably susceptible reactions [8] and Clostridium difficile the runs. It isn't prescribed in individuals with a background marked by extreme penicillin hypersensitivity [9]. It has all the earmarks of being moderately sheltered amid pregnancy [10]. It is in the third era cephalosporin class of meds. It works by disturbing the microscopic organisms' cell divider bringing about its demise. Cefixime, artificially (6R,7R)- 7-{[2-Amino-1,3-thiazol-4-yl)- 2-(carboxymethoxyimino)acetyl]amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4,2.0]oct-2-ene-2-carboxylic corrosive.

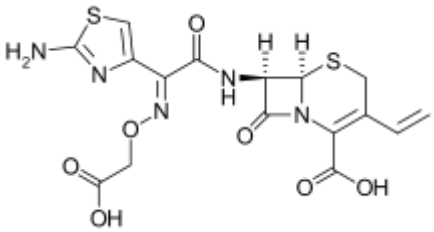

Fig1. Chemical structure of Cefixime

Lactobacillus is a virtuoso of Gram-positive, facultative anaerobic or microaerophilic [11], bar moulded, non-spore-shaping microbes [12]. They are a noteworthy piece of the lactic corrosive microscopic organisms gathering (i.e. they change over sugars to lactic corrosive). In people, they constitute a noteworthy segment of the micro biota [13] at various body destinations, for example, the stomach related framework, urinary framework [14] and genital framework [15]. In ladies of European family line, Lactobacillus species are typically a noteworthy piece of the vaginal micro biota [16]. Lactobacillus frames bio films in the vaginal and gut micro biota [17], enabling them to persevere amid brutal ecological conditions and keep up abundant populaces [18]. Lactobacillus shows a mutualistic association with the human body as it secures the host against potential

intrusions by pathogens [19], and thus, the host gives a wellspring of supplements [20]. Lactobacillus is the most widely recognized probiotic found in sustenance, for example, yogurt, and it is differing in its application to keep up human prosperity as it can help treat looseness of the bowels, vaginal contaminations and skin issue, for example, dermatitis [21].

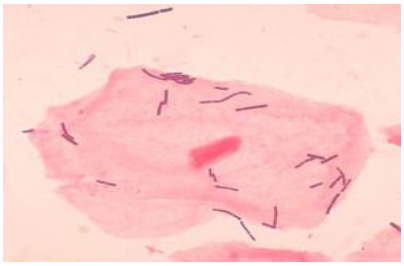

Fig2. Lactobacillus near a squamous epithelial cell

DRUGS:

Cefixime: (6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(carboxymethoxyimino)acetyl]amino]-3-ethenyl-8-oxo5-thial-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is an antibiotic used to treat infections by arresting the cell wall creation of bacteria. **Lactobacillus** is used in combination (cefic 50mg tablet)

MATERIALS AND METHODS

Chemicals:
Acetonitrile, octane-1-sulphonic acid, ortho phosphoric acid and water (HPLC grade) All active pharmaceutical ingredients (API) of cefixime as reference standards were used.

Equipment:
Water alliance-2695 chromatographic system consisting of quaternary pump, PDAdetector-2996 and chromatographic software Empower-2.0 was used.

Chromatographic conditions:
Chromatographic separation was carried out in isocratic mode at room temperature using a symmetry shield RP18 (150x4.6mm, 5µm) column. The mixture of buffer (3.0gm Octane-1-Sulphonic acid in 1lt water sonicated to dissolve adjusted the pH-2.5 with OPA): acetonitrile 60:40v/v at a flow rate of 1ml/min was used as a mobile phase. The injection volume was10µl and eluent was monitored at 282nm using PDA detector. The run time was 10min and each of the studied components was quantified by using total peak height.

Preparation of buffer P^H:
3.0gm of octane-1-sulphonic corrosive was weighed precisely and moved in to 1lt measuring glass and make the volume with water. The pH 2.5 was balanced with OPA.

Selection of Mobile Phase:
The mobile phase was set by injecting different ratios of buffer and acetonitrile. The selected mobile phase ratio was 60:40 v/v of buffer: ACN.

Selection of wavelength:
The absorption spectra of solution of each Cefixime and Lactobacillus were scanned over the range 200-400nm by using photodiode spectrophotometer and the spectra were recorded.

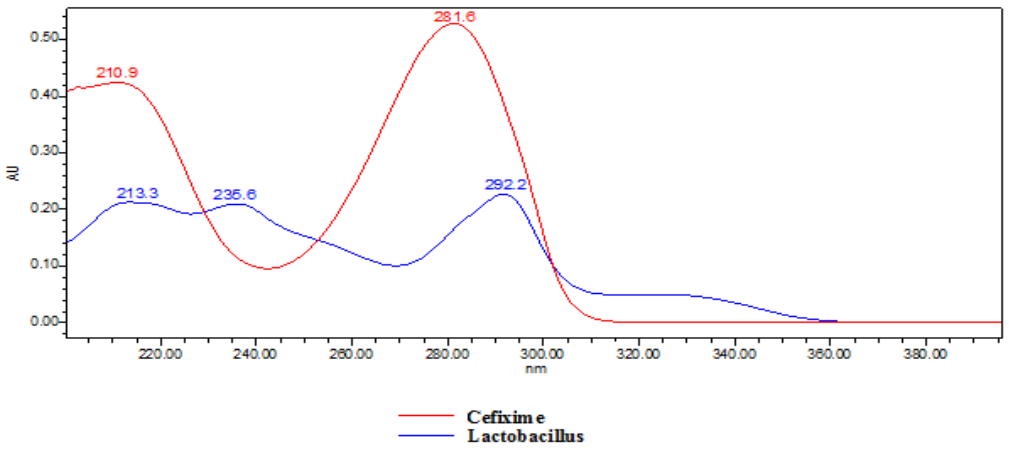

Fig3. PDA Spectrum for Cefixime and Lactobacillus

Preparation of Diluent:
Use Mobile phase as a diluents.

Preparation of standard stock solution:
Weighted about 66 mg of cefixime (working standard) and 20mg of lactobacillus (working standard) were accurately weighed and transferred to a 100ml volumetric flask. Then they were dissolved in 70ml diluent and sonicated for about 10min with intermittent shaking and diluted up to the mark with diluent.

Preparation of standard solution:
Transferred 5ml of standard stock solution were transferred into 50ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution:
Accurately weighed 250mg quantity of sample and was transferred into 100 ml volumetric flask and dissolved in 70 ml of diluent and sonicated to dissolve and diluted up to the mark with the diluent. Then, 5ml of the above solution was diluted with the 50ml diluent and was filtered through 0.45µ nylon syringe filter.

Procedure for Analysis:

An unfaltering pattern was recorded by the upgraded chromatographic conditions. It was balanced out for around 30min and progressive aliquots of the standard arrangement of a similar focus were infused and chromatogram was recorded until the point that the reproducibility of the pinnacle regions was agreeable. This method was continued utilizing the example arrangement with the goal that copy infusion of the example arrangement was sectioned by infusion of the standard arrangement. The reaction factor of the standard pinnacle and test top was acquired and the measure of each medication in the example was resolved. This method was rehashed six times. The grouping of each medication in the triple part measurement frame was computed utilizing the recipe,

Concentration of drug =

$$\frac{\text{Response factor of the sample x Concentration of standard}}{\text{Response factor of the standard}}$$

Validation Procedure:

The analytical method was validated as per ICH Q2(R1) [22] guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantization (LOQ), forced degradation and stability.

System Suitability:

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits.

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of other components (impurities, degradates or excepients), which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with Cefixime and Lactobacillus

Accuracy:

Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and amount of the drug present, percentage recovery and related standard deviation were calculated.

Precision:
Precision of an analytical method is the degree of agreement among individual test results. It was studied by analysis of multiple sampling of homogeneous sample. The precision of the present method was assessed in terms of repeatability, intra-day and inter day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

Linearity and range:
Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a definite range. The six series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression

equations were calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

LOD and LOQ:

LOD is the lowest amount of analyte in a sample that can be detected while LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy.

Robustness:

Its ability to remain unaffected by small but deliberate variations in method parameters. Robustness study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions such as flow rate (±0.2 ml/min), wavelength (±5nm), variation in PH (±0.5), organic content in the mobile phase (±2%). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

RESULTS AND DISCUSSION:

System Suitability:

Six imitate infusions of the blend containing 66 µg/ml of cefixime, 20 µg/ml of lactobacillus were evaluated to check the framework reasonableness.

Table 1: System suitability data

System suitability Parameter	Acceptance criteria	Drug Name	
		Cefixime	Lactobacillus
% RSD	NMT 2.0	0.5	0.2
USP Tailing	NMT 2.0	1.4	1.2
USP Plate count	NLT 3000	4558	5348

S.NO	Cefixime			Lactobacillus		
Inj	RT(min)	TP	TF	RT(min)	TP	TF
1	3.119	5285	0.68	7.196	4825	1.02
2	3.108	5255	0.14	7.184	4811	0.89
3	3.099	5268	0.48	7.182	4868	0.98
4	3.089	5261	0.85	7.186	4844	1.06
5	3.086	5290	0.85	7.184	4878	1.01
6	3.086	5224	0.87	7.178	4889	1.02

Table 2. System suitability data

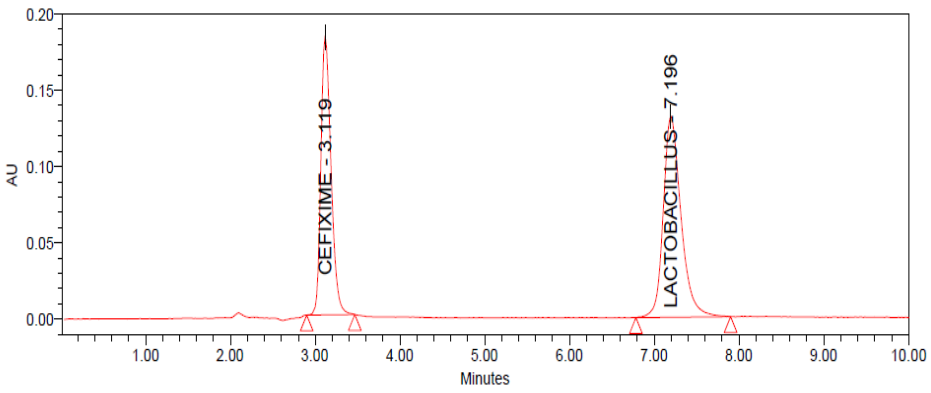

Fig 4. System suitability chromatogram

Specificity:

The interfering peaks cannot found during the blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

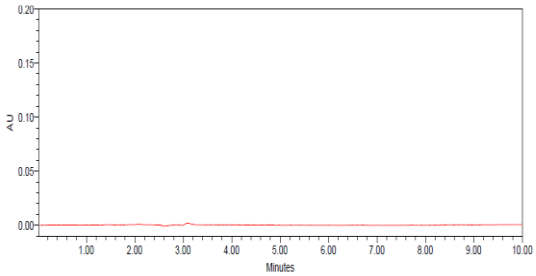

Fig 5: Chromatogram for Blank

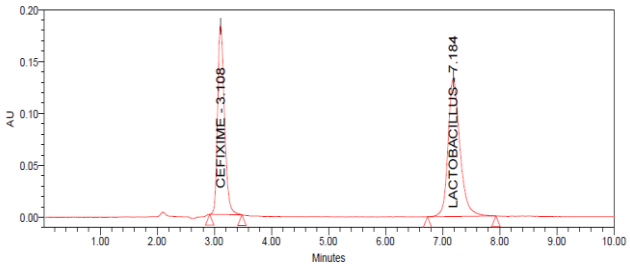

Fig 6: Chromatogram of Standard solution

SAMPLE:

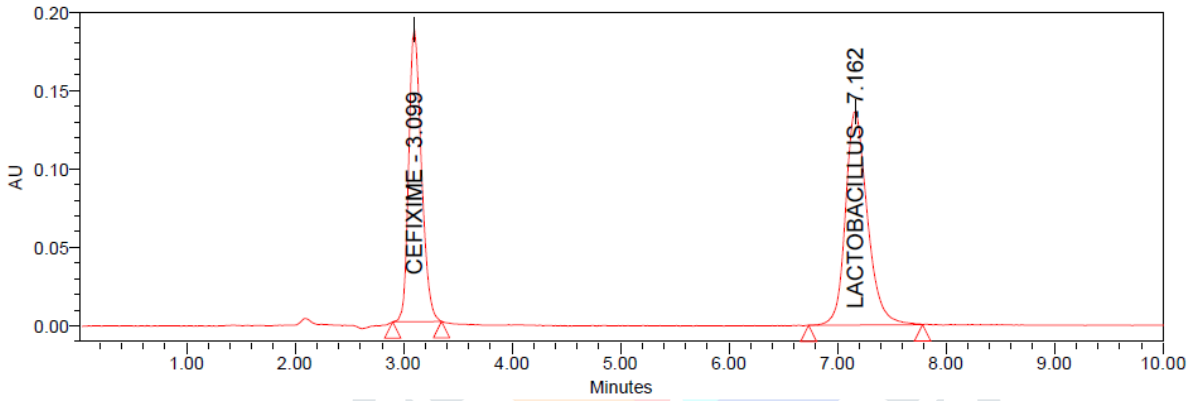

Fig 7: Chromatogram of Sample

Linearity:

Linearity was determined by plotting a calibration curve of peak area against their respective concentration. From this calibration curve it was found that the curve was linear in the range of 6.6-99 µg/ml for cefixime, 2-30 µg/ml for lactobacillus. The regression equations for calibrations curve was y=33269x-11362 (R2=0.9997) for cefixime, y=62486x-2570 (R2=0.9999) for lactobacillus respectively.

Table 3: Cefixime Linearity data

Linearity Level	Cefixime µg/ml	Area Counts
Linearity-1	6.6	217453
Linearity-2	16.5	539081
Linearity-3	33	1078162
Linearity-4	66	2156325
Linearity-5	82.5	2695406
Linearity-6	99	3334487
Correl Coeff		0.9997
Slope		33268.94
Intercept		-11362.23

Table 4: Lactobacillus Linearity data

Linearity Level	Lactobacillus µg/ml	Area Counts
Linearity-1	2	123582
Linearity-2	5	310395
Linearity-3	10	620791
Linearity-4	20	1241583
Linearity-5	25	1551978
Linearity-6	30	1882374
Correl Coeff		0.9999
Slope		62485.79
Intercept		-2569.99

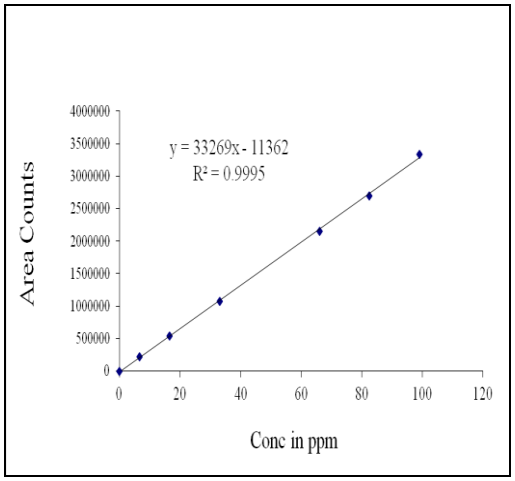

Fig 8. Linearity plot for cefixime

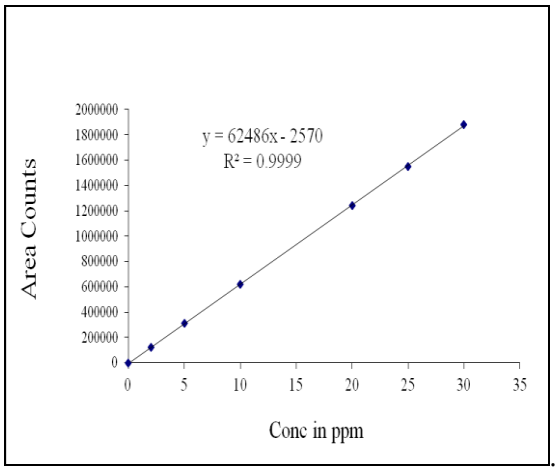

Fig. 9- Linearity plot for lactobacillus

Accuracy:

The accuracy of the method was performed by calculating the recovery experiments at three levels (50%, 100% and 150%). APIs with concentration 33, 66 and 99µg/ml of cefixime; 10, 20 and 30µg/ml of lactobacillus were prepared. The test solution was injected three times for each spike level and assay was performed as per the test method. The recovery results were close to 100% and also the RSD values were less than ±2%. The percentage recovery, mean and relative standard deviation were calculated. Recovery values demonstrated that the method was accurate within the desired range. The results are summarized below.

Accuracy	Amount of drug µg/ml	Recovery Solution (area) mAU	% drug Recovery
50%	33	1064058	100.1
100%	66	2160136	100.8
150%	99	3235394	100.3

Table 5 : Accuracy data for Cefixime

Accuracy	Amount of s drug µg/ml	Recovery Solution (area) mAU	% drug recovery
50%	10	617842	100.3
100%	20	1235214	100.2
150%	30	1875349	100.1

Table 6 : Accuracy data for Lactobacillus

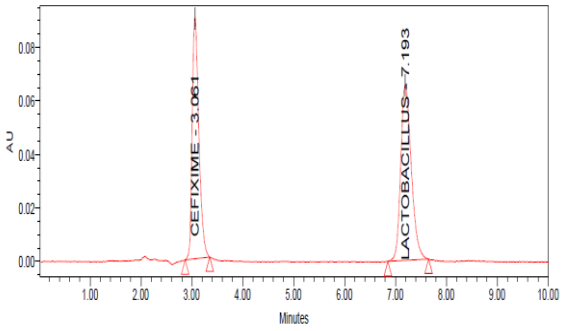

Fig.10-Chromatogram for Accuracy 50%

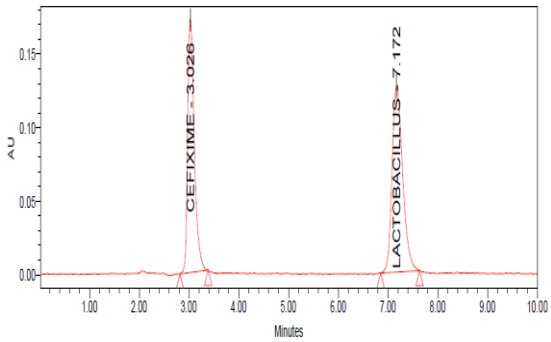

Fig.11-Chromatogram for Accuracy 100%

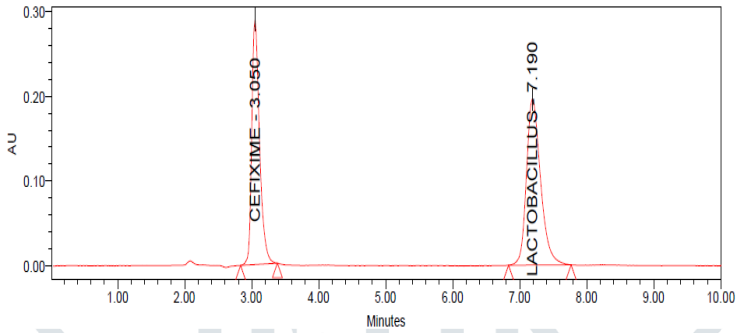

Fig.12-Chromatogram for Accuracy 150%

Precision:

Exactness of this technique was assessed in terms of intraday (repeatability) and intraday (middle of the road accuracy) varieties. The intraday contemplates were controlled by performing six rehashed examination of the example arrangement of cefixime and lactobacillus around the same time under the same trial conditions. The middle of the road accuracy of the technique was done in a similar research centre by concentrate the investigation with various expert and diverse instrument. The technique is profoundly exact as % RSD esteems were observed to be < 2%. Great recuperations (98 – 102%) of the medication were acquired at each additional focus, demonstrating that the technique was exact. The outcomes and chromatograms were outfitted beneath.

Cefixime			**Lactobacillus**	
S.No.	Rt	Area	Rt	Area
1	3.026	2159463	7.172	1289566
2	3.025	2163353	7.163	1286570
3	3.029	2157591	7.168	1264180
4	3.029	2161937	7.172	1292411
5	3.028	2137014	7.171	1275350
6	3.021	2135682	7.173	1270095
Avg	3.024	2135175	7.172	1275598
St dev	0.025	12153.79	0.031	14357.88
%RSD	1.05	0.8135	1.15	0.9585

Table 7: - Method precision data

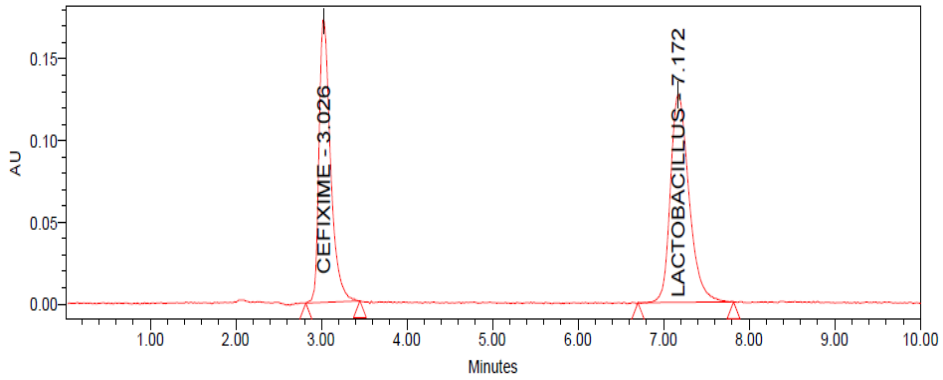

Fig.13-Chromatogram for Method Precision

Intermediate Precision:

SN.O	Area of Cefixime	Area of Lactobacillus
1	2174521	1238584
2	2148623	1284719
3	2162581	1240163
4	2158967	1264046
5	2169871	1271605
6	2146283	1285065
Mean	2168160	1254826
%RSD	0.41	0.86

Table 8. Intermediate precision data

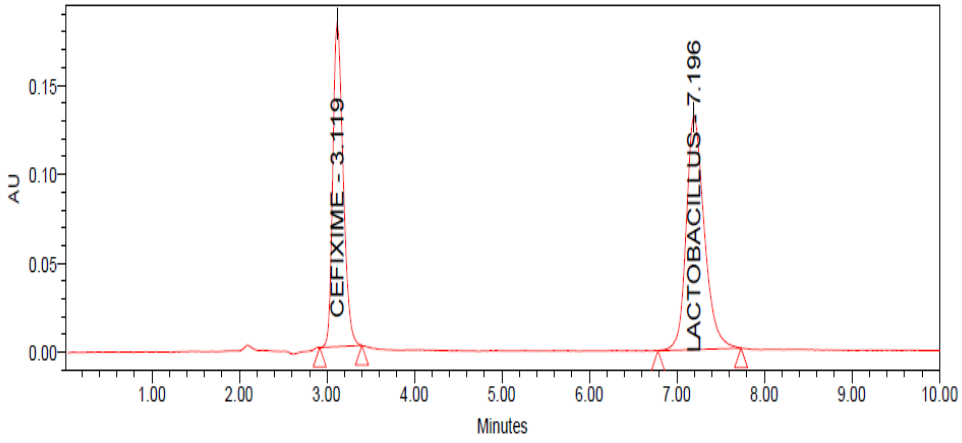

Fig 14. Chromatogram of Intermediate Precision

LOD & LOQ:

LOD and LOQ were separately determined by calibration curve method [23].The LOD values for cefixime and lactobacillus were found 0.06µg/ml and 0.2µg/ml respectively. The LOQ values were found to be 0.02µg/ml and 0.066µg/ml for cefixime and lactobacillus respectively.

S.NO	Sample name	LOD µg/ml	LOQ µg/ml
1.	Cefixime	0.06µg/ml	0.02µg/ml
2	Lactobacillus	0.2µg/ml	0.066 µg/ml

Table 9. Data of LOD and LOQ

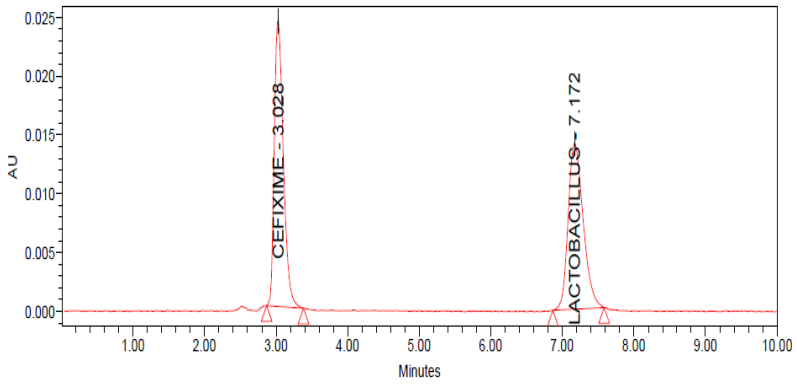

Fig.15-Chromatogram for LOD

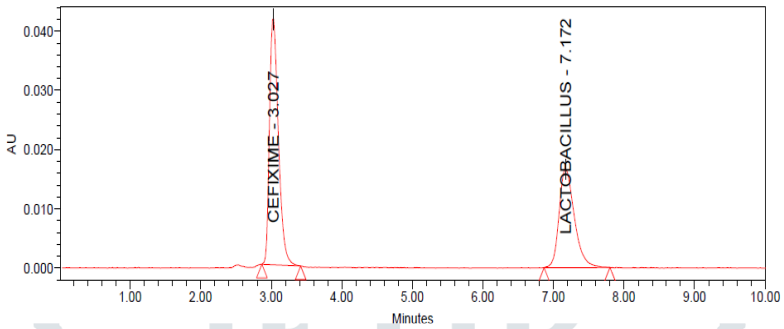

Fig.16-Chromatogram for LOQ

Robustness

As per ICH norms, small but deliberate variations were made in the method parameters such as change in the flow rate (±0.2), organic content in the mobile phase (±2%), wavelength of detection (±5) and pH (±0.5) to check the method capacity to remain unaffected. The robustness of the method was evaluated by observing the effect of the modified parameters on retention time, tailing factor, area, percentage content. The degree of reproducibility of the results which were obtained by small deliberate variations has proven that the method is robust.

Change in parameter	**% RSD for** Cefixime	**% RSD for** Lactobacillus
Flow (0.8 ml/min)	0.42	0.91
Flow (1.2 ml/min)	0.10	0.26
Organic phase composition (+2%)	0.08	0.18
Organic phase composition (-2%)	0.15	0.26
Wavelength (278 nm)	0.15	0.31
Wavelength (268 nm)	0.46	0.29
pH of the Buffer (+0.5)	0.28	0.45
pH of the Buffer (-0.5)	0.32	0.48

Table 10: Results of Robustness studies

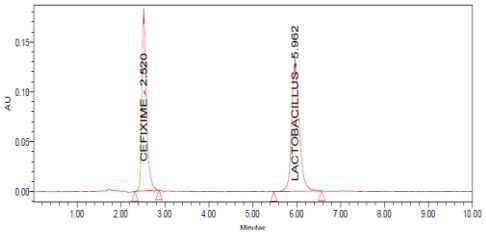

Fig.17-Chromatogram for Flow Plus

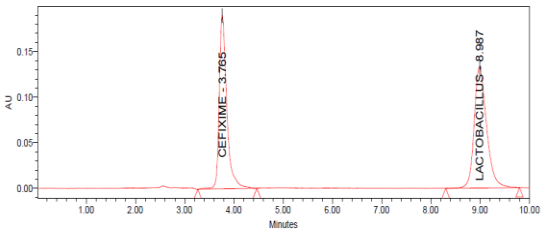

Fig.18-Chromatogram for Flow Minus

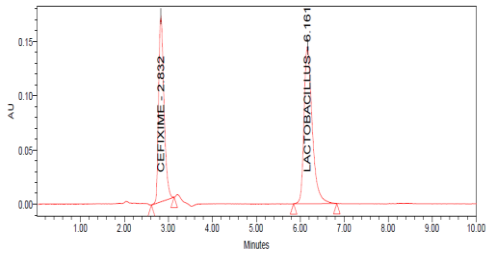

Fig.19-Chromatogram for Organic Plus

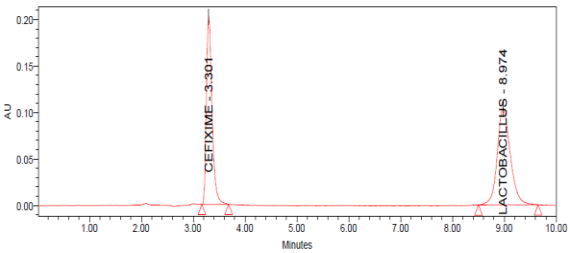

Fig.20. Chromatogram for Organic Minus

Assay:

Assay data for cefixime:

Assay	Assay		
	Area	**Average area**	**% Assay**
Preparation-1	2161408	2160586	100.3
Preparation-2	2159764		

Table 11. Assay data of cefixime

Assay data for lactobacillus:

Assay	Assay		
	Area	**Average Area**	**% Assay**
Preparation-1	1288068	1283182	100.5
Preparation-2	1278296		

Table 12. Assay data of Lactobacillus

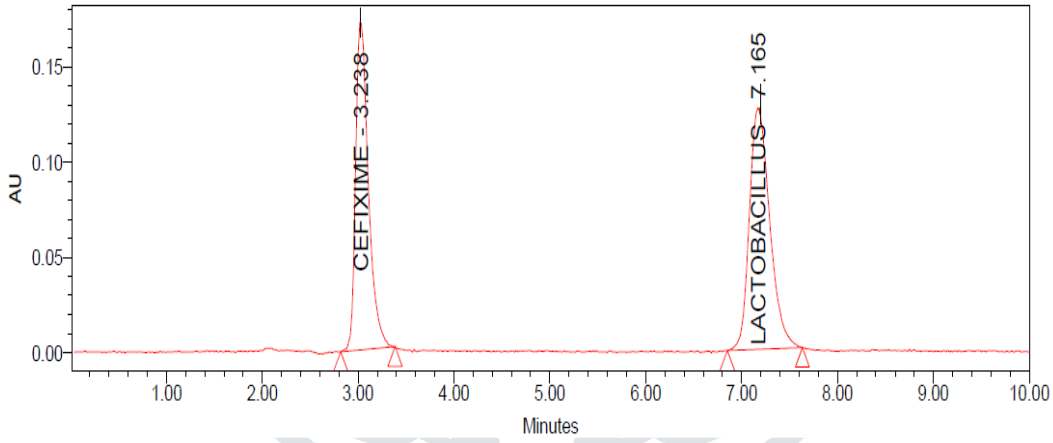

Fig 21. Chromatogram of Assay -1

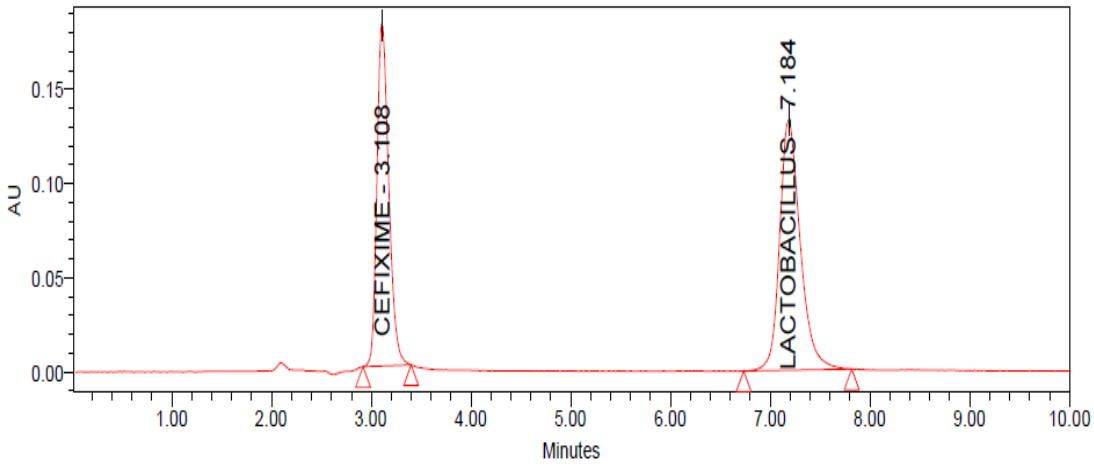

Fig 22. Chromatogram of Assay -2

Conclusion

In this investigation a novel, straight forward, quick, prudent, touchy and effectively accessible HPLC strategy was created for the concurrent assurance of cefixime and lactobacillus in mass and tablet dose frame. The principle points of interest of this technique over the beforehand announced HPLC singular strategies are its accessibility, shorter run times, low value, openness, affectability, unwavering quality and reproducibility. These properties are essential when an extensive number of tests are to be examined. The approval of the considerable number of parameters like linearity, precision, specificity, strength, dependability was done and observed to be inside the acknowledgment criteria. The RSD esteems for all parameters were observed to be less 2, which shows the legitimacy of technique and results gotten by this strategy are in reasonable assertion. So the proposed technique could be effectively connected for the normal examination and pharmaceutical details of cefixime and lactobacillus in quality control labs with no fundamental detachment.

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